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EFFECT OF VIBRATION UPON THE DENTAL PULP AND PERIOSTEUM OF WHITE RATS[1]

J. S. RESTARSKI, M.D.S.; D.D.S.[2]

From the Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.

Vibration in aircraft is one of the most pressing problems that the aeronautical engineer has to face (1). It is generally assumed that subjection to excessive vibration produces physiological and psychologic effects on the occupants of aircraft, although this has not been substantiated by experimental evidence (2).

The physiological effects of vibration on industrial workers has been studied by Koelsch (3). He found that workers with pneumatic drills and automatic hammers developed functional disturbances of the skin capillaries, and in some cases calcium deposits were formed in the joints and muscles. The vibrations studied by Koelsch were of sufficient magnitude to produce trauma; however, the amplitude experienced was not defined in his observations, and it is believed vibrations of such magnitude do not occur in aircraft.

Armstrong (2) stated, "The smallest amplitude of vibration which isperceptible is about 0.006 inch, and as the amplitude increases the sensation becomes more annoying and uncomfortable until at 0.05 inch amplitude the physiologic limit has been reached." Measurements of the maximum vibration (4) in the F4U Corsair of the control stick were gripped by the hand, registered amplitudes of from 0.05 to 0.07 inch at a frequency of 1000 CPM. The rudder pedals registered maximum amplitudes of 0.015 to 0.025 inch at 2000 CPM. Maximum vibrations occurring in the TB-1 Ventura were recorded in the nose compartment. The peaks were 0.044 inch at 2700 CPM, 0.025 inch at 3000 CPM, and 0.025 inch at 4300 CPM. According to Armstrong's criterion, only 2 of these measurements (0.05 and 0.07) are at amplitudes reaching the physiological limit.

Vibration of this amplitude and frequency does not occur at normal cruising speeds (4). On long runs the tendency of the pilot is to adjust the RPM of the engine to a "smooth-spot", and it is only at extremely high speeds when the plane is in combat, for instance, that maximum vibration is experienced.

A review of the literature on aviation medicine failed to reveal any information in regard to pathologic cellular changes in humans or laboratory animals following exposure to vibration as ordinarily experienced in aircraft. It was hoped that the experimental work presented here might provide some information concerning such changes following exposure of white rats to prolonged vibration of a known frequency and amplitude. Vibration of ± 0.05 inch ampli-

[1] The material in this article should be construed only as the personal opinion of the writer and not as representing the opinion of the Navy Department officially. Received for publication January 26, 1945.

[2] Lieutenant Commander, (DC), USN.

tude at 2600 CPM frequency was used as it is within the limits found in the F4U and TB-1 planes.

White rats were selected for the experiment because the rat incisor continues to grow throughout the life of the animal (5) and so is particularly favorable for metabolic studies. Parathyroidectomy, fluorosis, dietary deficiencies, etc. (6), and exposure to anoxia (7), all affect the dentin and enamel structure of the rat incisor. Such disturbance usually results in abnormalities of the series of concentric lamellae in the dentin surrounding the pulp canal. These lamellae appear in sections as incremental bands or rings which have an average width of

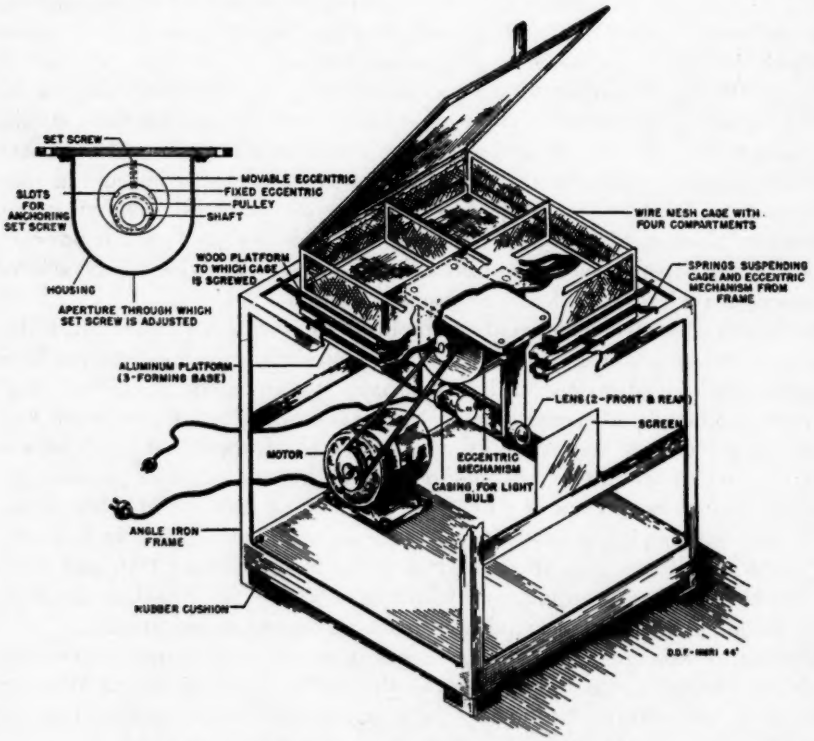

Figure 1. Vibrator used in experiments on rats

16 microns (the daily appositional growth of the dentin in rats) (8). Although the structure is uniform throughout, a part of this daily increment stains with eosin, and another part with hematoxylin. The basophilic portion is believed to be the section calcified during daylight hours.

PROCEDURES

Apparatus.—The vibrator (fig. 1) used in this experiment was constructed in the Naval Medical Research Institute technical shops. It consists of a vibrating platform suspended by springs from a heavy metal frame made of angle

iron. Vibration is produced by means of a movable eccentric rotor mounted under the platform. It is possible to vary the amplitude of vibration by changing the eccentricity of the rotor the desired degree from the concentric position. Rotation of the mechanism is accomplished by a "V" belt from a $\frac{1}{4}$ horsepower electric motor.

The amplitude of vibration is measured with an optical lever by projecting a beam of light from a fixed point through a lens assembly attached to the vibrating platform. For purposes of illustration, the distance between the light bulb and the lens is exaggerated in *fig. 1*. As the beam of light passes through the lens it is thrown on a calibrated screen (1.0 mm. equivalent to 0.01 inch amplitude) set at a measured distance from the lens. In this way it is possible to determine accurately the amplitude of vibration.

The frequency (CPM) is measured by a speed indicator applied directly to the shaft of the eccentric rotor.

Experiment.—The rats were subjected to vibration of ± 0.05 inch amplitude at 2600 cycles per minute, 8 hours daily, 6 days per week, for 28 days. During this time they had access to a complete diet.

The animals were segregated into male and female groups. Each vibrated rat was paired with a litter mate of the same sex as control. Of a large number used, 4 pairs the weights of which were within 20 grams of one another before the experiment and within 8 grams after the experiment, were selected for histologic study.

At the end of the vibration runs the rats were killed by intraperitoneal injection of sodium pentobarbital (Nembutal), perfused through the aorta with formalin-Zenker solution and portions of the maxillae and mandibles decalcified so as to yield longitudinal and transverse sections of the incisors, molars, and the temporomandibular articulation. The sections were stained with hematoxylin and eosin.

RESULTS

1. The total width of the dentin in transverse sections of the upper incisors (pulp canal to proximal at the middle one-third of the crown) of the rats exposed to vibration, averaged 156.0 to 245.5 microns; in the controls from 160.5 to 244.5 microns.

2. The total width of the measurable incremental rings in the dentin averaged from 292.5 to 352 microns in the vibrated rats, and 270 to 329 microns in the controls.

3. The number of measurable incremental rings in the dentin of the exposed rats averaged from 18 to 22; in the controls from 17 to 21. The width of the light portions of the incremental rings averaged from 9.2 to 11.4 microns in the vibrated rats, and 9.4 to 11.0 microns in the controls. The dark rings from the vibrated rats ranged from 4.6 to 6.9 microns; the controls 5.1 to 5.6 microns.

4. Histologic examination of the structural elements of the dentin, predentin, cementum, pulp tissue, odontoblasts, ameloblasts, enamel organ, labial alveolar periosteum, lingual periodontal membrane, and labial and lingual alveolar bone

failed to disclose any significant variation from normal. In similar structures of the molars, no changes were observed.

5. Similarly, the temporomandibular articulations in the animals exposed to vibration and those of the controls showed no abnormalities in the structure of the condyles. Among the structures examined were the newly calcified bone, calcified cartilage matrix, hypertrophic cartilage, vesicular cartilage cells, and hyaline cartilage cells.

The fibrous and synovial layers of the articular discs, and the structures of the mandibular fossae were normal.

SUMMARY

1. Exposure of white rats to vibration of ±0.05 inch amplitude at a frequency of 2600 cycles per minute, 8 hours daily, 6 days per week, for 28 days, did not affect the rate of growth of the incisor teeth, or the calcification of the dentin as shown by the constant average width and number of measurable dark plus light incremental rings in the dentin.

2. Histologic examination of the dentin, pulp, alveolar periosteum, alveolar bone, and the structures of the temporomandibular articulation failed to disclose any greater variation of cellular changes than occur normally.

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COMPARISON OF SPECIFIC GRAVITIES AND HARDNESS OF COHESIVE GOLD FOIL MALLETED WITH VARIOUS TYPES OF CONDENSORS[1]

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Because the gold foil filling will continue as a teaching requirement for its value in developing operative skill and because of its intrinsic value this investigation was undertaken. This study of the effects of various types of condensors on hardness and specific gravity also was stimulated by a dental student who counted 60 blows per pellet used by the author when placing a Class V restoration for him.

Rule (1), Ward (2), and Hawley (3) have reported studies on their phases of gold foil technic. Coleman (4) gave 6 different results obtained on restorations placed by 6 different operators malleting sample "restorations" on split steel molds. Using the same brand of gold foil and the same size molds, these operators produced specimens varying from 14.2 to 19.0 specific gravity. Black (5) stated that a gold foil restoration is not of real value unless it has a specific gravity of 14.0 or more.

PROCEDURE

Thirty-seven cohesive gold foil specimens were malleted, 28 in a bone-like specimen block and 9 in Class III cavities in ivorine teeth. The split block of bone-like material (manufactured by Baker and Co.) had a cavity cut in comparable shape to an occlusal cavity, 24.3 c.m.m. in size, in a molar tooth. Blocks were mounted on a wooden laboratory bench with an asbestos top which served as a sounding board. The 9 Class III cavities were cut in upper first incisors in a typodont mounted on the bench by a steel rod.

The plugger points were of approximately the same size but not identical because the different manufacturers had not standardized these instruments (Table I).

Pneumatic, automatic and hand mallets were used. The blows with the automatic and hand mallets were presumed to be maximal and might not be tolerated by all patients. With the pneumatic plugger light blows, readily tolerated, were used, as well as heavy blows (Table I). Straight and right angle hand pieces were used with the pneumatic plugger.

Neys No. ½ cohesive gold foil pellets were used for all preparations.

Specific gravity and Rockwell hardness measurements were made.[2]

[1] Received for publication December 15, 1944.

[2] These measurements were made by Dr. Paul F. Collins of Ohio State University.

TABLE I

Data on malleting

SPECIMEN NUMBER*	TYPE CONDENSOR†	CONDENSING POINT	TIME	NO. OF PELLETS	BLOWS PER NO. ½ PELLET	SPECIFIC GRAVITY	ROCKWELL HARDNESS‡
			min.				
1	Pneumatic	0.85 mm. sq-5-3	34	54	106	17.61	15T34
2	with light	"	45	50	114	17.44	15T41.5
3	blows—S	"	37	50	122	17.22	15T32
4		"	39	50	120	17.59	15T41
5	Hand mallet	0.85 mm. sq-6-3	40	40	80	18.4	15T40.5
6	"	"	45	40	80	18.6	15T47
7	"	"	40	40	80	18.5	15T29
8	"	"	40	40	80	18.6	15T35.5
9	Automatic	0.75 mm. sq-5-3	45	40	60	18.2	15T48
10	"	"	30	40	60	18.1	15T40
11	"	"	35	40	60	18.1	15T40.5
12	"	"	40	40	60	18	15T32
13	Pneumatic	0.78 mm. Round 5-3	35	40	120	17.7	15T33
14	with light	"	32	40	120	17.8	15T33.5
15	blows—S	"	32	40	120	17.8	15T31
16		"	30	40	120	17.6	15T37.5
17	Pneumatic	0.9 mm. sq. St. pt.	35	40	120	16.7	15T11.5
18	with light	"	30	40	120	16.5	15T23.5
19	blows—R	"	35	40	120	16.6	15T25.5
20		"	36	40	120	16.7	15T21
21	Pneumatic	0.8 mm. Round St. pt.	37	40	120	17.2	15T24
22	with light	"	29	40	120	17.2	15T32
23	blows—R	"	30	40	120	17.0	15T24
24		"	32	40	120	17.5	15T30
25	Pneumatic	0.78 mm. Round 5-3	38	40	150	18.4	15T40.5
26	with heavy	"	37	40	150	18.4	15T36
27	blows—S	"	36	40	150	18.9	15T44.5
28		"	32	40	150	18.7	15T50
29	Automatic	0.72 mm. Round pt. 5-3	90	75	40–60	18.0	15T50
30	"	"	70	50	40–60	17.2	15T55
31	"	"	70	59	40–60	17.7	15T35.5
32	Pneumatic	0.78 mm. Round 5-3	70	56	150	17.9	Split
33	with light	"	60	52	120–150	17.6	15T49
34	blows—S	"	50	46	120–150	18.2	15T40
35	Hand mallet	0.65 mm. Round pt. 6-3	75	53	60–90	18.5	15T35
36	"	"	70	52	60–90	18.6	15T36
37	"	"	60	52	60–90	18.2	?

* Specimens 1–28 were Class I cavities in a bone-like material: Specimens 29–37 were Class III cavities in upper first incisors.

† S—Straight handpiece. R—Right-angle handpiece.

‡ Rockwell hardness is average of tests on occlusal and pulpal or proximal and axial surfaces.

DATA

The data are given in Table I. Forty to 54 pellets were used in the 24.3 c.m.m. cavity, 40 in all except the 1st 4 instances with light blows of the pneumatic mallet of straight handpiece type. The number of pellets used in the Class III cavities were variable. The blows per pellet ranged from 40 to 60 with the automatic mallet on Class III restorations to 150 with the pneumatic straight handpiece mallet in the Class I type and Class III. The specific gravity was between 16.5 (right angle pneumatic) and 18.9 (straight handpiece pneumatic with heavy blows). The time consumed was from 30 minutes to 1½ hours but operations were sometimes interrupted by students permitted to observe the malleting.

DISCUSSION

The data indicate that pneumatic pluggers require less time for malleting and produce restorations of acceptable density. The density and hardness were increased with heavier blows, but the increase was beyond necessary standards (Table I, Specimens 13–16 and 25–28). Hand malleting did produce slightly denser foils than did any other method except heavy blows with the pneumatic mallet. Larger plugger points resulted in reduced density in specimens made under comparable conditions. (Table I, Specimens 17–20 and 21–24) and the right angle pneumatic plugger was less effective than the straight handpiece pneumatic plugger (Specimens 13–16 and 21–24). Specimens made with automatic plugger were comparable in density and hardness to those made with the pneumatic plugger. Clinical observation has indicated that the pneumatic plugger is easier on the patient and this study demonstrates that light blows with the pneumatic plugger will produce restorations with specific gravity of 16.5 to 18.6, above the acceptable standard (14.0).

The Rockwell hardness, as averaged from measurements on two surfaces, was not proportioned to the specific gravity.

SUMMARY

1. Restorations placed with pneumatic, automatic and hand condensors were all of acceptable density (14.0 sp. gr.).

2. Larger plugger points, lighter blows, and right angle pluggers produced restorations of lesser density.

3. Soft blows with the pneumatic condensor produced specimens of adequate density.

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CHANGES IN THE COMPOSITION AND SETTING CHARACTERISTICS OF PLASTER OF PARIS ON EXPOSURE TO HIGH HUMIDITY ATMOSPHERES[1]

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INTRODUCTION

Many users of plaster of Paris have observed that under certain conditions this material will deteriorate if its container is left open for long periods. Several instances have come under the notice of one of us (H. K. W.) concerning plasters whose setting characteristics altered very considerably on being transported in hessian bags (in the holds of cargo vessels) through the tropics. Furthermore, since the commencement of hostilities in tropical Pacific islands, it has not been an uncommon complaint of dental and surgical officers serving in those areas that their plasters "do not keep well." In some instances, it is reported that the material becomes excessively fast setting, but more commonly that it loses its ability to set properly or is "deadened." It would appear natural to seek some explanation of the observed phenomena in terms of the highly humid conditions persisting in these regions.

Almost simultaneously with the receipt of the first complaints from dental units operating in tropical areas, a paper describing the work of Farmer and Skinner (1) at Northwestern University was received in this Laboratory. This work, though confined to one "model" plaster, was sufficiently comprehensive to throw much light on the troubles which were being reported. These investigators claimed that plasters may be stored safely in atmospheres having relative humidities below approximately 70%, though troublesome fluctuations of the setting time may occur with variation in the humidity from day to day. Plasters stored at a relative humidity greater than 70% ultimately absorb considerable water and become "deadened" and can be reclaimed only by calcination. In experiments carried out with an atmosphere of 100% relative humidity, Farmer and Skinner observed their samples to exhibit, first of all, a progressive increase in setting rate when mixed with water, but later to become "deadened." This observation was explained as follows: In the early stages gypsum nuclei form. These act collectively as an accelerator, but later the gypsum becomes sufficient in amount to produce a protective sheath around the plaster particles and thus retard the set abnormally. The present work was initiated to check some of the observations of Farmer and Skinner, and to provide data of a more extensive

[1] This work was made possible by a grant from the National Health and Medical Research Council of Australia. Received for publication January 3, 1945.

[2] Proof read by Dr. F. A. Peyton, University of Michigan, Ann Arbor, Michigan.

nature and covering a wider range of materials. With this information it was hoped to throw light on some points which seemed a little obscure or problematical in the explanations put forward by these workers.

EXPERIMENTAL PROCEDURES

The preliminary experiments included tests under varying conditions on several dental plasters and "quick setting stones." This work confirmed in general that of Farmer and Skinner, and, furthermore, demonstrated that "quick setting stones," as well as ordinary laboratory plasters, would deteriorate on exposure to high humidities. It was obvious, however, that the different samples absorbed water at markedly different rates. In the light of the preliminary experiments, 3 representative plasters were chosen for more extensive investigation: A. An imported plaster which absorbed water rapidly; B. An Australian plaster which absorbed water moderately quickly; C. An Australian plaster which absorbed water slowly. Subsequent to the work on samples A, B, and C, additional experiments were carried out on an English plaster of high purity (plaster D), in the hope of throwing light on points which arose in the earlier investigations. All the plasters were at least 2 years old before the commencement of the tests, and plasters A and D had already passed through the tropics, D being trans-shipped at Ceylon.

Having in mind the work of Farmer and Skinner (1), only two humidities were utilized:

(1) 75% relative humidity at 27°C (81–82°F) which is representative of average humidity and temperature conditions encountered in tropical climates[3] and below which there appears to be little alteration in the setting time and characteristics of most dental plasters after moderately prolonged exposures.

(2) 100% relative humidity at 27°C. This latter humidity, although higher

[3] Some values for humidity and temperature in tropical areas in and near Australia are as follows:

REGION	RANGE OF MONTHLY AVERAGE RELATIVE HUMIDITIES %	RANGE OF MONTHLY AVERAGE TEMPERATURES
		degrees Fahrenheit
Solomons, New Caledonia Area	60–90	60–90
Celebes and Eastern Part of the N. E. I.	65–90	80–85
Port Moresby (Papua)	70–80	70–90
Other Parts of Papua	60–95	70–90
Rabaul (New Britain)	70–80	70–90
Darwin (Australia)	45–80	75–90
Northern Queensland (Coastal)	65–85	65–95

than most average atmospheric conditions, is approached quite often for short periods in certain tropical areas. In addition, the effects given at this humidity are greatly accelerated and thus more readily observable.

The plaster was spread out in Petri dishes which were kept in cabinets of constant temperature and humidity, and samples were removed at regular intervals and tested for water content and setting time.

DETERMINATION OF WATER

During the major part of this work, the hygroscopic or physically absorbed water was estimated by exposing the plaster over concentrated sulphuric acid for 3 to 4 days and weighing. The remaining chemically combined water was then determined by heating the material at 300–400°C for 4 hours in an electric furnace. However, more recently it was discovered that the process of drying over concentrated sulphuric acid would not only remove the hygroscopic water but also some of the "combined" water. In view of this difficulty, it is now realized that it would have been more satisfactory to determine the hygroscopic water by oven drying at 45°C.

As an illustration of the unsuitability of the H_2SO_4 desiccation technique the following data may be quoted: A sample of plaster B, which had been exposed to a 75% relative humidity atmosphere for about 250 days and contained a total of 10% water, was left over concentrated sulphuric acid for a prolonged period (10 weeks). During this time it lost water at a constant rate up to the 6th week (loss about 9.2%), and thereafter lost water at a much reduced rate until the 10th week, when the loss had reached 9.6%. On further exposure over the acid, the weight remained constant.

The ability of plaster of Paris to lose "combined" water on exposure to very dry atmospheres may be explained in terms of the modern conception of the structure of the hemihydrates of $CaSO_4$. Kelley, Southard and Anderson(2) have postulated that the water in these crystals exists in "channels" throughout the lattice. Furthermore, the attractive forces between the water molecules and the calcium and sulphate ions are much smaller than those between the calcium and sulphate ions themselves. The combination of these factors permits changes in the water content of the hemihydrates without essential variation in their lattice structures. It is thought that the uncertainty in the determination of hygroscopic water does not apply when complete hydration of the material to the dehydrate has taken place.

In view of the doubt as to the accuracy of the earlier figures on hygroscopic water, the total water content (that is, chemically combined and hygroscopic) has been shown in the graphs of the data up to the stage at which complete hydration had taken place. Thereafter, the combined water alone has been shown. In this connection, it should be noted that before complete hydration has occurred, the amount of hygroscopic water present is of real significance since it is probably this water which is utilized in the building up of the dehydrate crystal lattice. When complete hydration has occurred, it has no longer any significance, and hence for this and the above reason has been omitted.

DETERMINATION OF SETTING TIME

The initial setting time was determined using a $\frac{1}{4}$-pound Gillmore needle. This indicates the time (from the commencement of mixing) at which the surface layers of the plaster mix develop a compressive strength of approximately 46 pounds per square inch ($\frac{1}{4}$ pound acting through a needle $\frac{1}{12}$ in. in diameter). The plaster was added to the water over a period of $\frac{1}{2}$ minute and spatulation was continued for another $\frac{1}{2}$ minute. A constant water/plaster ratio (1:2) was used in all cases. The Gillmore test, although satisfactory in the initial states of the experiments, is not so reliable for indicating the progress of gypsum crystallization when the plaster has previously absorbed an appreciable quantity of water from the atmosphere. This has been shown by using the Cloez (temperature rise) method which indicates the end of the period of major crystallization. (See discussion for elaboration of this point.)

EXPERIMENTAL RESULTS AND DISCUSSION

The most significant results are summarized in *figs. 1, 2, 3 and 4*. The graphs show both how the water content and the setting time changed on exposure of the materials to the 75% and 100% relative humidity atmospheres.

It is interesting to observe certain similarities in the shapes of the 75% and 100% relative humidity curves in *figs. 1 and 2* and less noticeably in *fig. 3* (where the experiments were not carried on for such a long period). In each case there is an initial period with a very slow increase in the water content, and this is followed by a marked rise in the case of the 100% relative humidity curve and a less marked but still quite definite increase in the case of the 75% relative curve. This increase in the water content continues until sufficient water has been absorbed to make possible complete hydration to the dehydrate. Although the 75% relative humidity curves do not show an amount absorbed equal to that at 100% water was still being absorbed, and no doubt complete hydration would ultimately have occurred.

Fig. 4 summarizes the work which was carried out on an English plaster of high purity, in order to provide more detailed information about the behaviour of the material during the early stages of the exposure to 100% relative humidity, and also to investigate the effect of various calcination treatments. The curves marked I in the graph indicate the variation in water content and setting time of the original plaster. Curves marked II give results on the plaster which, as preliminary treatment, had been maintained at 120°C for 4 hours. It contained 6.7% water at the end of this period, showing a decrease of about 1% in water content, and its setting time had been increased from 4½ minutes to 22 minutes, indicating that probably the most important effect of the heating at this temperature was to calcine any $CaSO_4 \cdot 2H_2O$ which was present and which would act as an accelerator to the setting of the plaster. On exposure to the humid atmosphere, a gradual increase in water content occurred over the first few days and a corresponding decrease in setting time took place. This was followed by a sudden increase in the water content, which had the effect of markedly increasing the setting time as determined by the Gillmore needle technique. The curves

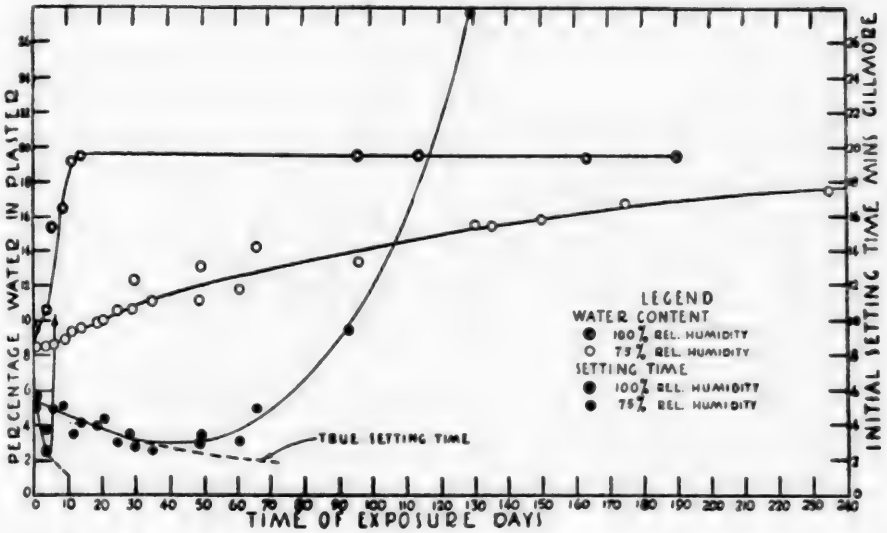


Fig. 1. Variation in water content and Gillmore setting time of plaster A on exposure to 75% and 100% relative humidities at 27°C.

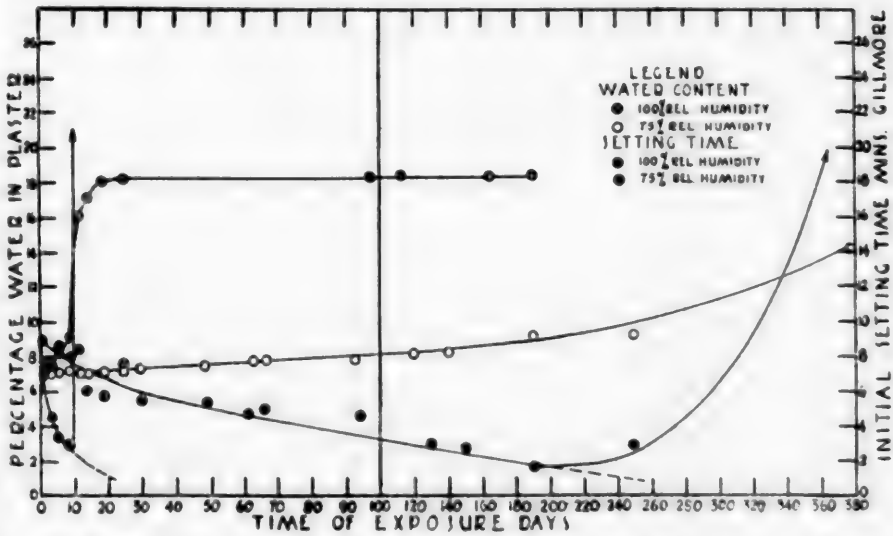


Fig. 2. Variation in water content and setting time of plaster B on exposure to 75% and 100% relative humidities. Note change in scale of abcissae after 100 days.

III show the behaviour of the plaster after being heated to 180°–190°C for 4 hours in an electric furnace. It then contained only 0.7% water, thus indicating that most of the hemihydrate (as well as any dehydrate present) had lost its

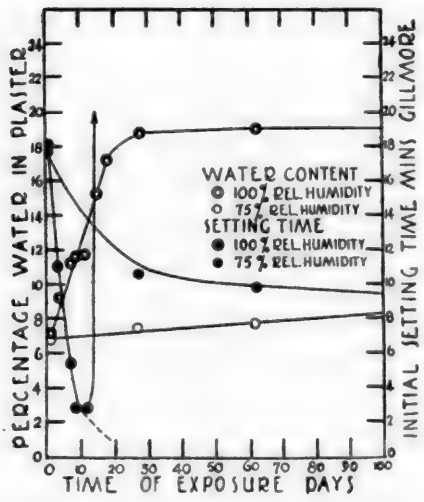


Fig. 3

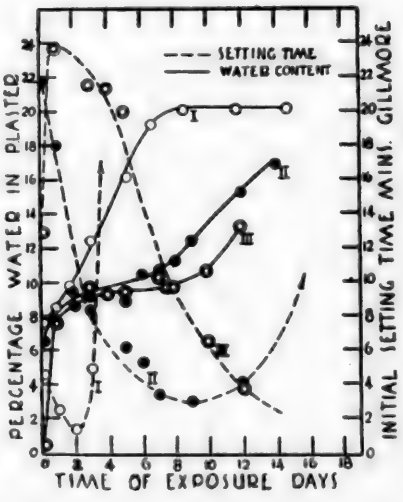


Fig. 4

Fig. 3. Variation in water content and setting time of plaster C on exposure to 75% and 100% relative humidites.

Fig. 4. Curves showing water absorption and setting time variation of an English plaster of high purity on exposure to 100% relative humidity. I. Plaster as taken from container. II. Sample had previously been heated to 120–130°C for four hours. III. Sample had previously been heated to 180–190°C for four hours.

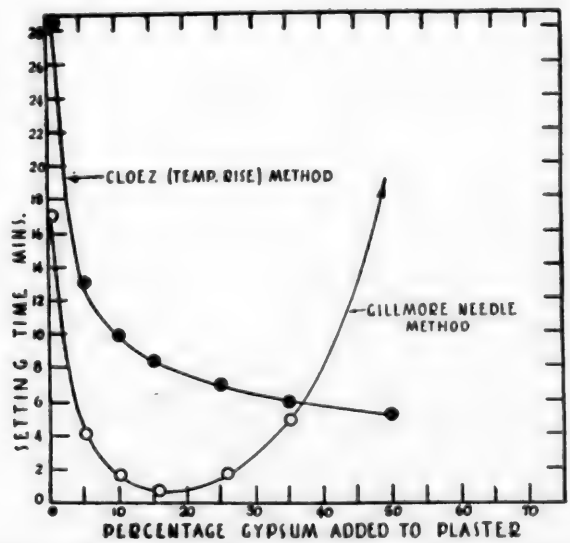


Fig. 5. Comparison of Gillmore initial set and Cloez (temperature rise) setting time with varying proportions of finely powdered gypsum added to the plaster.

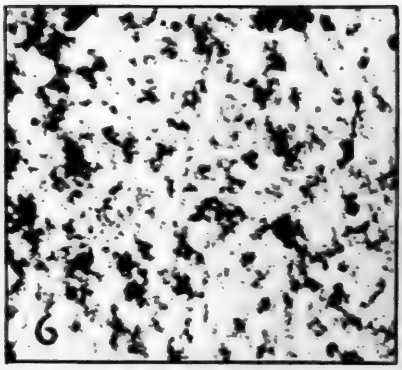
water. After 12 hours' exposure to 100% relative humidity, the plaster had absorbed sufficient water to give the hemihydrate, and this was accompanied by an increase in setting time from 13 to 24 minutes. Thereafter, on further exposure, the material behaved in a very similar way to that of curve II.

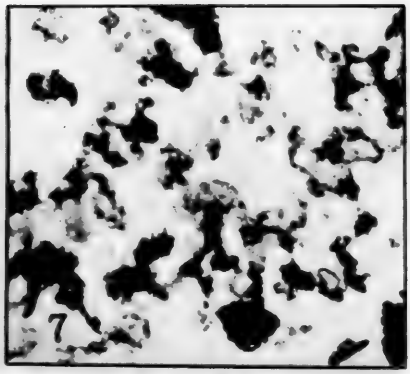
It will be seen that for each plaster, as the percentage of water absorbed from the atmosphere increases, the Gillmore initial setting time decreases up to a certain stage and then increases again, ultimately becoming so great that the plaster would be described as being "deadened." If, however, the Cloez (temperature rise) method is utilized, the setting time is found to decrease continuously with increase in "atmospheric" hydration of the sample. The difference between the results given by the 2 methods is well illustrated in *fig. 5*, which gives data of experiments in which varying percentages of finely ground gypsum were added to plaster and the setting time determined by both Gillmore and Cloez methods. The latter method is undoubtedly a truer indication of the existence and progress of gypsum crystallization, and care must be exercised in interpreting the Gillmore results after the minimum in the setting time curve is reached.

Two factors are of importance in explaining the increase in setting time as shown by the Gillmore test after a certain stage: (a) As the amount of gypsum in the plaster increases, the setting rate is so greatly accelerated that the crystal aggregations already forming in the mix are broken up during spatulation, and thus play little or no effective part in strengthening the mass subsequently. (b) The gypsum which is formed by absorption of moisture from the atmosphere serves to weaken the mix, since it is "inert" from the point of view of the setting of the plaster (other than acting as an accelerator). In view of these two factors, it is clear that if a high percentage of gypsum is present in the plaster, the mix may never become strong enough to withstand the pressure of the Gillmore needle. The hardening which occurs is mainly that due to drying out of the mix. These observations eliminate the necessity for the postulation of Farmer and Skinner (1) that the setting of the exposed plaster is finally retarded abnormally by "protective sheaths of gypsum."

Two additional facts which may be observed from a close examination of the data are of interest: (a) On exposure to high humidities, plasters take up moisture at different rates. For example, at 100% relative humidity plaster A (*fig. 1*) was fully hydrated after about 10–12 days, whereas plaster B (*fig. 2*) required about 20 days and plaster C (*fig. 3*) required about 30 days' exposure. The discrepancy in rates of absorption of water is even more evident in the 75% relative humidity results. (b) Those plasters "reacting" quickly (those absorbing moisture quickly) have initially a short setting time, and those "reacting" slowly have initially a relatively long setting time.

These observations may be explained on the assumption that the dehydrate acts as a catalyser, not only for the normal setting of plaster, but also for the absorption of water from a moist atmosphere. The fast setting (and fast "reacting") plasters have been found to contain an abnormally high percentage of gypsum (nearly 10% in the case of plaster A as against about 2.5% in the case





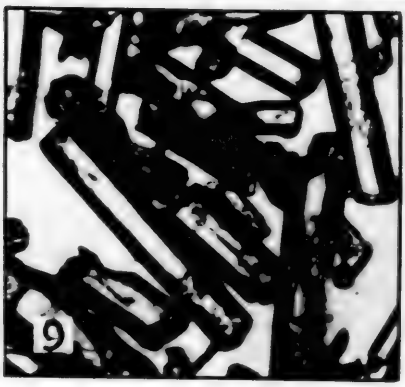


Fig. 6. Particles of plaster B as taken from drum ×250.

Fig. 7.—Sample of same plaster after exposure to 100% relative humidity for 12 days, ×250. Slides for *figs. 6 and 7* prepared by making suspension of the material in absolute alcohol and evaporating off the alcohol.

Fig. 8a. Portion of a gypsum crystal (selenite) ×700. *fig. 8b.* – Same as *fig. 8a* after calcination ×700. *Fig. 8c.* Same as *fig. 8b* after exposure to 100% relative humidity for 48 hours ×700.

Fig. 9. Crystals of hemihydrate ×250.

Fig. 10. Similar crystals after exposure to 100% relative humidity for 48 hours ×250. (Illustrations in each case obtained with transmitted illumination.)

of plaster B, and less than 1% in plaster C). The work summarized in *fig. 4* also illustrates this point well. Destroying the gypsum present in the plaster had the double effect of increasing the setting time greatly and of slowing down the rate at which the material absorbed moisture from a humid atmosphere. Thus it seems very probable that the dehydrate crystals act as nuclei for further crystallization on exposure of the plaster to moist atmospheric conditions, and hence, as hydration proceeds we should get a marked increase in the *rate* of hydration, as indicated by a sudden rise in the water content curve. This process might be compared to that of autocatalysis—that is, the product of a reaction acting as a catalyser in bringing about further reaction. This is shown to a remarkable extent by the 100% relative humidity curves, and to a lesser extent by the 75% relative humidity curves. However, it seems unlikely that a plaster originally slow setting and which had been made fast setting by the addition of either nonhygroscopic salts or gypsum crystals would absorb water quickly, since these substances would not be so effective catalytically as the very finely divided gypsum remaining in the hemihydrate particles as a result of incomplete calcination. In this regard, no means were available for distinguishing between gypsum remaining after calcination and that which might have been added by the manufacturer as an accelerator in plasters A, B or C.

MICROSCOPIC EXAMINATION OF THE MATERIALS

In order to determine what changes took place in the microscopic appearance of the plasters on exposure to high humidity atmospheres, some considerable time was devoted to observation of test samples at various magnifications. Initially, samples were selected from the Petri dishes at successive intervals, and an attempt was made to detect crystal growth in the particles of the material, analysis having indicated that progressive hydration was occurring. However, the fact that the same particles were not observed during the successive periods made comparison of the kind desired very difficult. This work did show a tendency on the part of the particles to "hang together," a tendency also observable in the bulk state. This phenomenon is one cause of the characteristic known as "clogginess" in plasters. The change in the microscopic appearance is shown in *figs. 6 and 7*. The only conclusion that could be drawn from this portion of the work was that if gypsum crystals did form then they must be very small.

To obviate the above mentioned difficulties, another series of observatons was carried out starting with a small crystal of selenite. In *fig. 8a* an edge of the original crystal is shown at high magnification. *Fig. 8b* depicts the same edge after the crystal had been subjected to calcination at a temperature of 130–150°C for 2 hours. It is noticeable that considerable checking or cracking of the crystal has occurred, although the general contours remain almost unchanged. *Fig. 8c* shows the crystal after exposure for 48 hours to an atmosphere of 100% relative humidity. The planes of the original selenite crystal have completely vanished and have been replaced by masses of small randomly oriented crystals. The edge of the particle also exhibits some crystal growth. *Figs. 9 and 10* show

similar changes occurring on crystals of the hemihydrate grown from 50 to 60% nitric acid according to a method outlined by Caspari (3). *Fig. 9* shows the original crystals and *fig. 10* a similar group of crystals after exposure to 100% relative humidity for 48 hours. The growth of needle-like gypsum crystals is particularly evident in this case.

CONCLUSION

1. All plasters and "quick-setting stones" deteriorate in humidities of the order of 75%. Such highly humid conditions are likely to be encountered in many places; in certain tropical areas, values as high as 90–95% are frequently attained. Under these conditions, the greatest care should be exercised at all times to minimize contact of plaster of Paris and its related products (e.g., casting investments) with the atmosphere.

2. The rate at which the characteristics of these materials change as a result of such exposure may differ markedly.

3. Those plasters initially fast setting are more likely to deteriorate rapidly on exposure to high humidity atmospheres. The rate of absorption of moisture at any particular humidity appears to depend principally on the quantity of dehydrate left in the plaster after calcination, or subsequently formed therein by absorption of moisture from the atmosphere.

4. Initially, on exposure to high humidities, there is an increase in the setting rate followed by an apparent decrease as determined by the Gillmore needle method. Actually, however, the increased percentage of "inert" material present, does not permit the mix to become sufficiently strong (after spatulation for the standard time) to resist indentation by the $\frac{1}{4}$-pound Gillmore needle. The hardening which ultimately occurs is more probably the result of the drying out of the mix than of crystallization of gypsum.

SUMMARY

Samples of dental plasters were exposed to atmospheres of 75% and 100% relative humidity for prolonged periods and tested at regular intervals. Both analytical and microscopic evidence showed that the plasters absorbed water from the moist atmosphere and underwent hydration to $CaSO_4 \cdot 2H_2O$. The rate of absorption of moisture at any particular humidity appears to depend mainly on the quantity of dehydrate already present in the plaster. The setting rate increases continuously on exposure, ultimately making the plaster unusable.

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A STUDY OF THE ANGLE OF THE MANDIBLE[1]

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The angle of the mandible lies between a line along the posterior border of the ramus and the horizontal plane on which the lower border of the body of the mandible rests in its natural position (1). It may be measured on a special hinged board which has a protractor fixed at the hinge. The angle is determined after adjusting the hinged piece of the board so that it touches the condyle and the most prominent point along the posterior border of the ramus near the angle. Another method of measuring the angle is to make an outline drawing of the posterior border of the ramus and lower border of the body of the mandible with a dioptrograph, and to read the angle after drawing in the corresponding straight lines; this is essentially what is done when determining the angle from a radiograph.

MATERIAL

The following material was used in this investigation: (1) A number of mandibles from an anthropological collection in the department, used by permission of Professor M. R. Drennan. (2) Another series of mandibles from a collection of more recent dissecting room material. (3) A large number of angle of mandible measurements obtained from radiographs in the Radiological Department of the Groote Schuur Hospital.

Altogether, the data cover measurements from 262 individuals, 127 skulls and 135 radiographs of the heads of living persons. From a racial point of view the mandible data considered here are very mixed. Taking the hospital records and the Anatomy Department entries as a criterion, the individuals are classified as follows: Cape colored 56%; European 37%; natives 7%. The Cape colored have resulted from an admixture of European, Hottentot, Bushman and Negro blood, in varying proportions, but from a social standpoint pure-bred Indians, Malays and some Chinese are also included amongst them. The figure for Europeans (37%) is probably too high, as there is a tendency among this section of the population to call themselves European whenever possible. The so-called Natives are the Bantu.

DIFFERENCES BETWEEN THE RIGHT AND LEFT ANGLES

For purposes of this study the left angles of the mandibles have been measured, and the same procedure has been followed where the angles have been obtained from the radiographs of living persons.

The 2 sides of the mandible are not always perfectly symmetrical. Apart

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from possible slight differences due to inherent general asymmetry, there are differences caused by irregular increases in thickness along the lower border of the mandible or along the posterior border of the ramus. Such increases in thickness reflect differences in the state of the teeth on the 2 sides, or differences in the development of the muscles of mastication, associated with acquired preferential habits of chewing on one or the other side. A specially powerful masseter or medial pterygoid of one or the other side can cause a roughening or hyperostosis near the angle, and this in turn causes slight differences in the lines of the lower or posterior borders. However, such differences between the right and left angles seldom exceed 3 degrees.

In a series of 50 unselected mandibles the results shown in Table I were obtained. This table gives the differences between the right and left angles without stating which side was the larger. If the differences between the right and left sides in this group of 50 mandibles are expressed as R-L angles, the mean difference was found to be −.18°, the standard deviation 1.99°, and the standard error of the

TABLE I

Differences in measurements of right and left angles in 50 mandibles

	NUMBERS
Angles equal on the two sides	12
Difference of 1° between right and left angles	14
Difference of 2° between right and left angles	10
Difference of 3° between right and left angles	11
Difference of 4° between right and left angles	2
Difference of 5° between right and left angles	1
Total	50

mean .282°. On the assumption that the right and left sides of the mandible tend towards symmetry and that the true mean difference between the 2 sides would be 0, the observed mean difference −.18° falls well within the range of the standard error of the mean. The calculations show that the differences between the right and left angles found in this group were fortuitous, and that there is no tendency for the angle of one side to be greater or smaller than that of the other side.

There is, therefore, every justification for using the measurements of one side consistently, in this case the left. The left side has the additional advantage of following the anatomical and anthropological convention of representing the left side of the norma lateralis of the skull.

AGE CHANGES IN THE ANGLE OF THE MANDIBLE

The increase in the angle of the mandible which occurs in older people is described in text-books of anatomy as a corollary of advancing age, usually being accompanied by the loss of teeth. Observers who have studied this so-called

age change of the bone from a dental point of view, stress the importance of the loss of teeth rather than of advancing age. For instance, Sprawson (4) stated that "the ages at which the angle of the mandible increases and at which the loss of alveolar bone occurs depends to a large extent on whether and when the teeth were extracted, or if they were lost from parodontal disease; whether dentures had been worn and whether these functioned efficiently."

An analysis of the present observations supports the view that there is no increase of the angle in the mandibles of older people, provided sufficient teeth remain in the jaws to ensure a 'bite'. Data from 3 age-groups were collected, 6–21 years, 25–45 years, and 50–76 years, limiting this series to individuals with good teeth or a sufficient number of teeth to hold the jaws apart when the mouth was closed. The results are shown graphically in *fig. 1*.

Fifty observations each were obtained for the 6–21 years and the 25–45 years age groups, but only forty observations for the 50–76 years, and these were arranged in order from the smallest to the largest angles. It was difficult to collect sufficient data for the age-group 50–76 years. Among the class of people covered by this investigation there are comparatively few individuals at this age who still possess their own teeth.

In all 3 age-groups there is a wide range of variation within the normal, from 110 to 140 degrees, with occasional instances outside these ranges. It will be noted that approximately one-half of the angle measurements were obtained from the bones, and the other half from radiographs. Before combining these data and using them together in the subsequent analysis, the reliability of the radiological method of measuring the angles of the mandibles was established. Several mandibles were radiographed in the lateral position with such amounts of variation from the true lateral as commonly occur in practice when taking lateral radiographs of patients' heads. The angles of these mandibles were then measured on the special osteometric board. The differences in the angle readings between the direct and the radiological method were so slight as to be negligible.

The histograms of the several age groups (*fig. 1*) indicate certain differences. If the 25–45 years group (histogram *a*) is compared with that of the 6–21 years group (histogram *c*), the respective arithmetic means are 125.4° and 128.3°. The standard error of the differences between these two groups is 1.44°, the difference between the means 2.9°, with a critical ratio of 2.0. The difference between the arithmetic means is just twice the standard error of the differences. This shows that the result is probably significant, and that it is unlikely to be a chance finding. Presumably the tendency towards the larger angles of childhood has not yet disappeared.

On the other hand, if the 25–45 years group (histogram *a*) is compared with the 50–76 years group (histogram *b*), the respective arithmetic means are 125.4° and 123.5°. The standard error of the differences between these 2 groups is 1.51°, the difference between the 2 means 1.9°, with a critical ratio of 1.3. This cannot be considered as significant and is within the range of a fortuitous result.

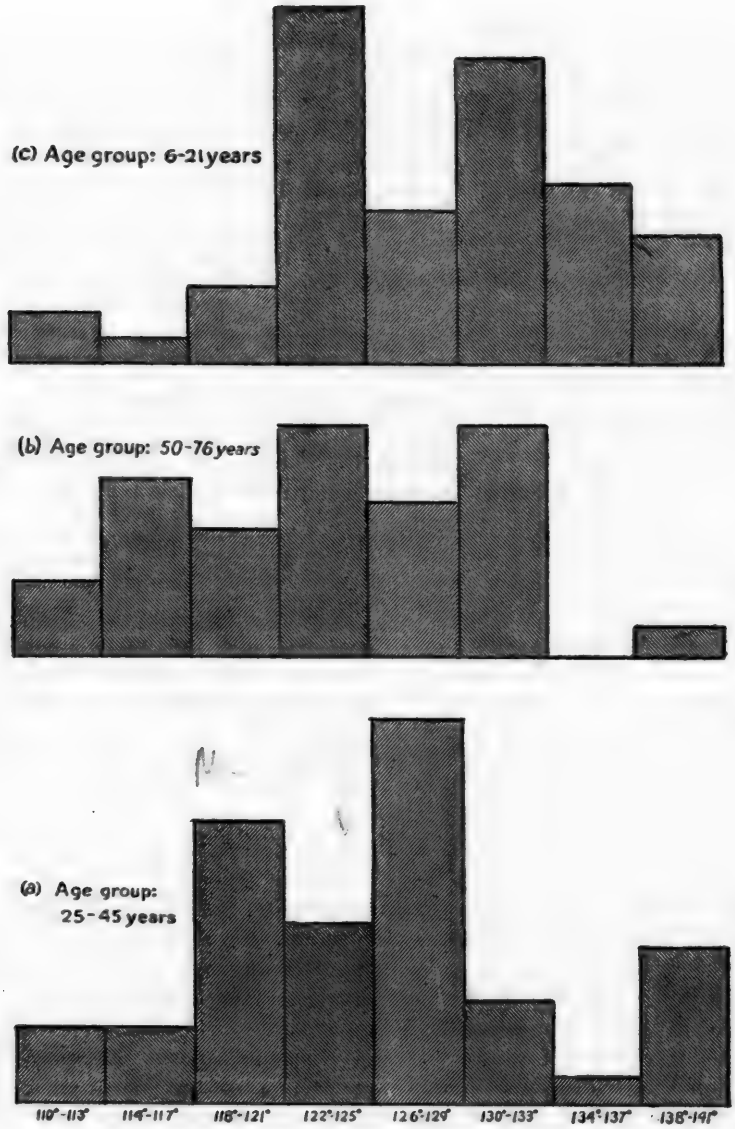

Fig. 1. Histograms showing frequency distribution of angle of the mandible measurements in different age groups, in individuals with teeth.

Statistically the frequency distribution of the angle of mandible measurements is a similar one in the adult and the older group. The analysis of the findings shows that in persons who retain their teeth there is no tendency towards an enlargement of the angle with advancing years.

ANGLE AND LENGTH OF THE MANDIBLE CONSIDERED IN RELATION TO THE CONDITION OF THE TEETH

In sharp contrast to the histograms of *fig. 1* is the histogram of *fig. 2* showing the frequency distribution of the angle measurements in a group of 50 edentulous people in which the age factor was not considered. There were no angles less than 119° and there was an accumulation of angles of 130° or more. Many individuals in the edentulous group were persons under 30 years; the youngest was 17, and the average age of the whole group was 47.6 years.

If the 25–45 years age group (*fig. 1*, histogram *a*) is compared with the edentulous group (*fig. 2*), the significance of the difference in the angles of the mandible is very obvious. The respective arithmetic means are 125.4° and 130.5°. The standard error of difference is 1.4°, the difference between the arithmetic means 5.1°, with the highly significant critical ratio of 3.6. The probability that this result is fortuitous is .00016.

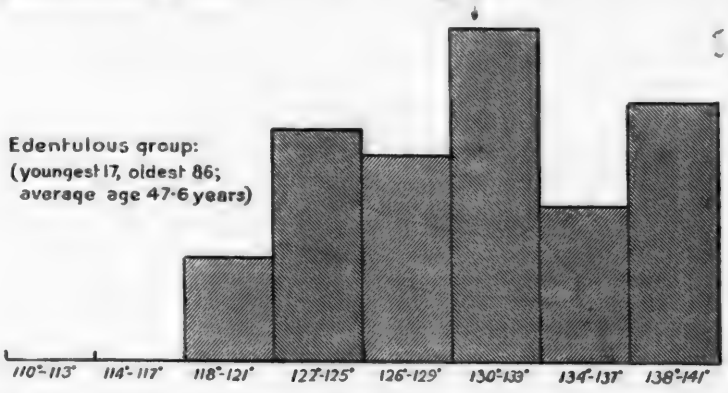

Fig. 2. Histogram showing frequency distribution of angle of mandible measurements in fifty edentulous persons.

It could not be decided how many of these edentulous persons had artificial teeth, but it is obvious that a good many of them must have possessed dentures. The radiographs from which about half the observations in *fig. 2* were made, had been taken in order to diagnose various conditions of the head without reference to the teeth; the patient removes his dentures as a routine before an X-ray is taken.

DISCUSSION

Stein (5) has illustrated the mechanics of the mandible with a complete dentition by comparing it to a loaded beam, compression in the region of the angle being effected by the masseter and medial pterygoid muscles against a resistance offered by the condyles and the teeth.

It is easy to see what happens after complete extraction of the teeth, when dentures are not fitted. The mandible is afforded an extra 'play' in its swing on the condyles by the gap which has been made. An effort to oppose the jaws

now brings the alveolar mucosa of the mandible up against the alveolar mucosa of the incisor region of the maxilla, before the biting surfaces of the molar portions of each bone can come into contact. This makes chewing difficult. As the alveolar borders become absorbed, as they invariably do under such circumstances, the 'play' becomes still greater, and the difficulty mentioned is accentuated.

The action of the masseter and medial pterygoid muscles at the angle is now no longer opposed by the contact of the whole biting surface of the mandible with that of the maxilla, but only by contact of the alveolar tissues near the front of the mouth. The muscles of mastication will thereafter tend to mould the mandible in such a way as to restore the parallelism of the biting surfaces, and this must involve an increase in the angle of the mandible.

The data show an increase of 5.1° when the arithmetic mean of the angles of 50 edentulous mandibles was compared with that of a similar group of 50 mandibles with teeth. The mechanics of the increase in the angle is clearly seen in the lateral radiograph of the face of a woman, aged 30, whose teeth had

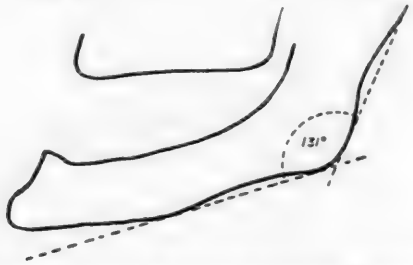

Fig. 3. Tracing from radiograph of the head region of a woman, aged 30, who had been edentulous for 18 months, and had not worn dentures.

been extracted 18 months previous to the time of taking the X-ray, and who had not been fitted with artificial teeth (*fig. 3*); also in the skull of a dissecting-room subject, a man, aged 70 (*fig. 4*). In this skull (*fig. 4*) there is a complete absence of the usual 'fit' of the mandible to the maxilla, showing that he did not have dentures and that the gum margins were habitually brought into contact. The mental foramen is close to the upper border on each side and faces upwards, showing that the alveolar borders had become absorbed.

Length of mandible. A corollary to any increase in the angle of the mandible is an increase in its length. The length of the mandible, as an anthropometric measurement, is the maximum distance between the most backward projecting points on the 2 condyles and the most forward projecting points of the chin in the midline, the measurement being taken with the mandible resting in its natural position on a horizontal surface (*fig. 4, a-b*).

The increase in length is reflected in a protrusion of the chin. It is well illustrated by *fig. 5*, in which an edentulous mandible is viewed from below in its natural position against the base of the skull. The protrusion has destroyed the good 'fit' of the biting surfaces, not only in the incisor region, but also further

back, where a broader portion of the mandible has come to lie opposite the back part of the maxilla. The alveolar margin of the maxilla thus becomes clearly visible within the framework of the mandible. This is not so when the angle of the mandible has been preserved and the mandible has not elongated, in spite of the loss of teeth, as in the case shown in *fig. 6*; here the outline of the hard palate is covered by that of the mandible.

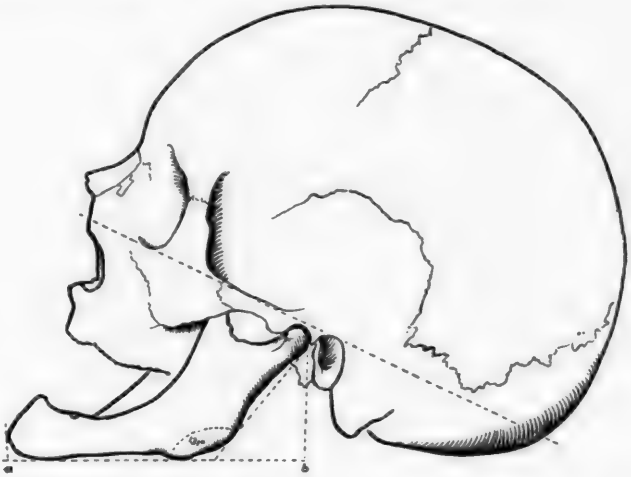

Fig. 4. The left lateral aspect of the skull of a man, aged 70, who had not worn dentures

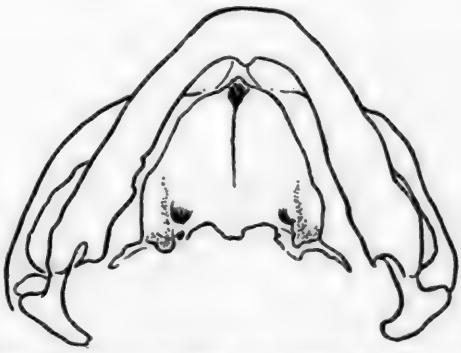

Fig. 5. Mandible and hard palate of the skull in fig. 4, seen from below

Effect of dentures. If dentures are fitted immediately after extraction, they naturally abolish the 'play' due to loss of teeth, and will thus for the time being maintain the normal mechanism of the mandible. In most cases, however, the dentures fail to preserve the alveolar borders, which are gradually absorbed; dentures fitted immediately, generally have to be changed after a few months. This absorption gives rise to precisely the same sort of 'play' as was described

in the case of the mandible without dentures, and its adjustment requires a similar increase in mandibular angle. From the examination of the radiographs of a number of people with well-fitting dentures I have obtained the impression that the angle is usually large and the body of the mandible narrow, indicating resorption of the alveolar borders.

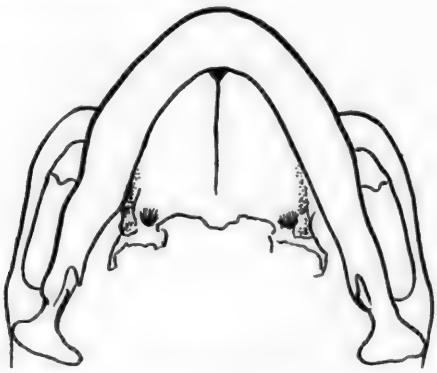

Fig. 6. Mandible and hard palate of skull in fig. 7, seen from below

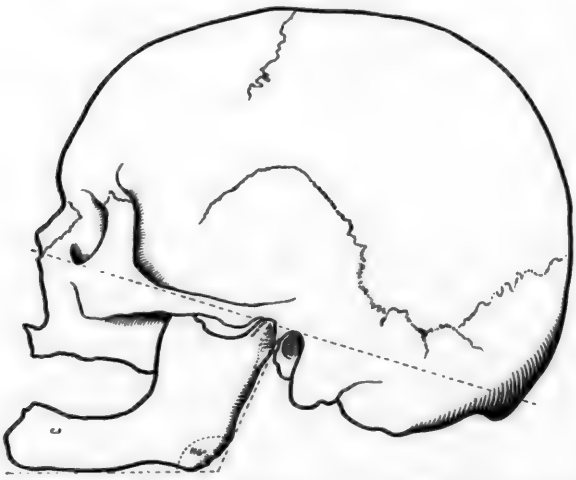

Fig. 7. Left lateral aspect of skull of a woman, aged 70, who had worn dentures

Very occasionally well-fitting artificial teeth preserve the alveolar borders, the angle does not widen and there is no lengthening. Such a case is illustrated in *fig. 7*, a sketch of the skull from a dissecting-room subject, a woman, aged 70. There is a good 'fit' of the mandible to the maxilla, a small angle (116°), and the alveolar border is preserved as shown by the position of the mental foramen 8 mm. below the upper border. *Fig. 7* should be contrasted with *fig. 4*.

The success of artificial teeth in preserving the angle of the mandible depends primarily, therefore, on the preservation of the alveolar borders. Saunders (3) suggested that this was so, and advocated extremely careful extraction to avoid alveolar fractures, with immediate fitting of dentures before any modification of the alveolar borders could take place. I cannot agree, however, with Rogers and Applebaum (2) who imply that the fitting of dentures will habitually preserve the angle, showing a skull very similar to that seen in *fig.* 7 to support their view; alveolar absorption takes place too commonly for this to occur habitually. In this connection one must mention that alveolar absorption or its prevention is just as important in the maxilla as in the mandible.

It will be noted that in the presence of dentures the distortion of the mandible is likely to proceed only to a degree necessitated by the absorption of the alveolar borders. The increase in the angle and the amount of lengthening is not likely, on the average, to be as great as when dentures are not available. The demonstration of this point was not possible in the present study, owing to the difficulty of knowing which edentulous subjects had worn dentures and which not. From what has been said above, however, it seems likely that the increase in the angle of the edentulous group would have been still more striking, had the exclusion of those with dentures been possible.

SUMMARY AND CONCLUSIONS

The author has studied the angle of mandible measurements of 127 skulls, supplemented by the angle measurements obtained from the radiographs of 135 living persons, a total of 262 measurements. The changes in the angles were investigated from the point of view of age and the condition of the teeth, and the following conclusions were reached:—

(1) After arranging the angle observations into 3 appropriate age groups and constructing suitable histograms of frequency distribution, the author showed conclusively that the angle does not increase with advancing age per se.

(2) A comparable histogram of a group of edentulous individuals of all ages, showed a definite increase in the angle which must be attributed to the loss of the teeth.

(3) The mechanics of the moulding of the edentulous mandible by the muscles of mastication is explained. In this connection the author stresses the extremely important rôle played by the absorption or non-absorption of the alveolar borders in the mandible and the maxillae.

(4) Immediate denture service sometimes prevents the absorption of the alveolar borders and preserves the angle. More often, the alveolar borders are nevertheless absorbed, the angle becomes wider and the mandible lengthens.

The author wishes to thank Dr. J. M. Grieve, Head of the Radiological Department of the Groote Schuur Hospital, for permission to make use of X-ray films from the Department, and Mr. J. de Villiers for collecting and making radiological angle measurements in the various age-groups. Further, to thank Mr. J. G. Taylor, of the Psychology Department, for the help received in the statistical analysis of the numerical data. Also to gratefully acknowledge the help received from a Research Grant of the University of Cape Town.

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THE ROLE OF ACTINOMYCES ISRAELI IN SALIVARY CALCULUS FORMATION[1]

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Many theories have been advanced as explanations of salivary calculus formation. Experimental work has been rather scanty. Prinz (1) believed calculus formed by (a) the loss of CO_2 from stagnant saliva, raising its pH and favoring calcium salt precipitation and (b) precipitation of colloid pellicles onto the surface of the teeth. He produced precipitation in *vitro* with saliva and "artificial saliva" but did not mention the role of bacteria.

Bulleid (10) suggested the importance of leptotrichia. Naeslund (2) found numerous bacteria, chiefly leptothrix and actinomyces, in calculus. Some investigators now consider that both organisms were variants of *Actinomyces israeli*. Naeslund demonstrated that loss of CO_2 and protein decomposition were of little importance under intraoral conditions in calculus formation and suggested that the actinomyces may have a chemical influence by proteolysis and a physical significance in providing a tenacious framework for deposition of calcium salts. Smith (4) and Adamson (5) demonstrated alkaline phosphatase in the mouth and opened discussion of its role. Bibby (6) demonstrated the importance of bacteria in calculus retention and incriminated leptotrichia, rather than actinomyces. Murray and Pincus (7) and Zander (8) investigated the actions of phosphatase and demonstrated its presence by chemical methods. Rosebury and Karshan's review (3) and Rosebury's discussion (9) have suggested the need for determination of the presence or absence of alkaline phosphatase in the organisms present in calculus.

In order to demonstrate the role of *A. israeli* in calculus formation, to confirm or refute Naeslund's work, and to correlate the production of calcular deposits in vitro with the presence of bacterial-produced or bacterial-free phosphatase, the following study was undertaken.

EXPERIMENTAL

Six strains of *A. israeli*, identified by cultural and morphological characteristics (12) were studied. Of these, 2 strains were isolated from cervico-facial actinomycosis and 4 from gingival lesions other than actinomycosis.

1. *Proteolytic capacity.* A. Liquefaction of gelatin. 6 tubes containing 3 cc. of autoclaved saliva and enough nutrient gelatin to make 9 cc. were inoculated

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with the different actinomyces strains. Growth occurred but the gelatin was not liquefied.

B. Liquefaction of Loeffler's slants. Loeffler's slants, inoculated with 5 of the strains of actinomyces, showed growth but no liquefaction.

C. Tests for production of protein decomposition. Six strains inoculated into tryptone broth plus 3 cc. of autoclaved saliva and one in dextrose infusion broth showed moderate growth but no indole production by Ehrlich's tests. The Foster strain, cultivated in iron agar medium, showed no H_2S production.

2. *Alkalinizing ability.* All 6 strains were inoculated into test tubes containing each of the following media: tryptone broth plus autoclaved saliva (3 cc.) to make 9 cc.; dextrose infusion broth plus autoclaved saliva (3 cc. to make 9 cc., nutrient gelatin plus autoclaved saliva (3 cc.); tryptone broth plus 1 cc. of 0.2% autoclaved calcium chloride; and infusion broth plus 1 cc. of 0.2% autoclaved calcium chloride. All were incubated anaerobically for 6 days and then refrigerated for 2 to 3 weeks. The pH was determined by the glass electrode. Table I shows that in only 2 instances slight increases in pH occurred in the infusion broth plus saliva. In many instances, the acidity of the medium was increased when dextrose was added to the above broth.

3. *Ability to precipitate calcium from a solution not containing phosphates.* Three tubes of infusion broth plus 1 cc. of 0.2% autoclaved $CaCl_2$ were inoculated with *A israeli* and 2 tubes served as controls. After moderate growth was noted the contents of the tubes were centrifuged and the precipitate thoroughly washed and dissolved in acetic acid. Potassium oxalate revealed only faint traces of calcium in controls and tests.

4. *Ability to produce alkaline phosphatase.* The tissue staining method for phosphatase (Kabat and Furth (13)), Landow, Kabat and Newman (14) proved unsatisfactory. The cultural plate-indicator method of Bray and King (15) was used with the Foster and TC strains. The former was strongly positive while the TC reacted to a lesser degree. Calculus and exudate from cases suspected of being actinomycosis were also tested by streaking the materials on Dorset's egg medium. These gave positive results. Further investigation was halted by lack of phenolphthalein phosphate.

DISCUSSION

Naeslund (2) believed actinomyces of importance in precipitating and binding calcium salts in calculus. The data presented agree with Naeslund as to the role of the organisms but offer an entirely different concept of the mechanism involved. While Naeslund assumed a bacterial proteolysis, the strains of *A. israeli* studied did not show such activity. Naeslund (16), himself, had been unable to demonstrate the proteolysis conclusively. The results of our investigation fail to show an increase in alkalinity of the medium, a function Naeslund had ascribed to this microorganism. In this study, the *A. israeli* failed to bring about precipitation of calcium from solutions that did not contain organic phosphate, although Bulleid (11, 12) showed precipitation of calcium as phosphates

TABLE I

pH of anaerobically incubated and refrigerated cultures of A. israeli

STRAIN	GROWTH AND COLONY FORM	pH
	Tryptone-Saliva Media pH 7.6	
Reddy	moderate, discrete pinheads	7.5
"	slight, pinpoints	7.6
IM	abundant, pinpoints	7.7
"	slight, pinpoints	7.5
Foster	moderate, pinheads	7.1
"	moderate, pinpoints	7.4
1597	slight, hazy	7.5
1597	moderate, pinheads	7.7
TC	abundant, pinheads	6.8
"	abundant, pinpoints	7.0
CW	moderate, pinheads	7.3
"	slight, hazy	7.6
Control	none	7.7
"	none	7.6
	Gelatin-Saliva Media pH 7.6–7.8	
Reddy	slight, pinheads	7.8
IM	doubtful, hazy	7.7
Foster	slight, pinpoints	7.2
1597	no growth	7.8
TC	abundant, pinpoints	7.4
CW	doubtful, hazy	7.7
Control	none	7.6
"	none	7.8
	Dextrose Infusion Broth—Saliva Media pH 7.2	
Reddy	abundant, spreading	4.6
IM	doubtful, cloudy tube	4.8
Foster	scant, spreading	6.1
1597	abundant, spreading	4.7
TC	abundant, spreading	4.8
CW	moderate, spreading	4.8
Control	none	7.2
"	hazy, particles on tube	7.3

TABLE—*Continued*

STRAIN	GROWTH AND COLONY FORM	pH
Dextrose Infusion Broth-Saliva Media pH 8.8		
Reddy	moderate, hazy	8.5
IM	doubtful	8.6
Foster	doubtful	8.5
1597	doubtful	8.5
TC	abundant, pinpoints	8.5
CW	doubtful	8.8
Control	cloudy	8.8
"	cloudy	8.8
Infusion Broth-Saliva Media pH 7.3		
Reddy	moderate	7.6
IM	scant, spreading	7.5
Foster	scant, pinheads	7.0
1597	moderate, pinpoints	7.3
TC	moderate, spreading	6.5
CW	doubtful, hazy	7.2
Control	none	7.3
"	none	7.3
Tryptone Broth-$CaCl_2$ pH 7.1		
Reddy	moderate, pinpoints	6.5
IM	none	7.0
Foster	none	7.1
1597	moderate, spreading	7.0
TC	moderate, pinpoints	5.7
CW	none	7.0
Control	none	7.1
"	none	7.1

from a solution containing both elements and leptotrichia. The present study demonstrated that *A. israeli* can liberate phosphate from organic chemical combination, presumably by the action of alkaline phosphatase.

If, *A. israeli* contains sufficient quantities of alkaline phosphatase to cause calcium to precipitate as phosphate, then this organism can produce salivary calculus by its metabolic activity and it can cause it to adhere to teeth by its ability to cling to a smooth surface. Further work to correlate the presence of salivary calculus with various strains of *A. israeli* is indicated.

SUMMARY

A. israeli, as indicated by study of 6 strains, was unable to decompose protein material into such simpler compounds as indole or H_2S; also was not able to raise

the alkalinity of media or precipitate calcium from an inorganic calcium chloride solution that did not contain phosphate ions.

A. israeli, as indicated by study of 6 strains, contains alkaline phosphatase and, therefore, may cause precipitation of calcium phosphates from the saliva and initiate calculus formation.

The author wishes to thank Dr. Theodor Rosebury for his aid in planning and supervising this study. Dr. Henry Bartels for his assistance in writing the report, and Dr. Elvin Kabat for training in the technic for demonstration of phosphatase and use of his laboratory and materials.

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TRANSPLANTATION OF DEVELOPING TOOTH GERMS IN THE MANDIBLE OF THE CAT

A Roentgenologic Study[1]

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INTRODUCTION

In 1940, we began a series of experiments to test the capacity of the tooth germ to substitute for the tooth of another animal. We have removed developing permanent teeth of young cats, replaced these in each case with a tooth germ from other, usually younger cats, and recorded the progress of the transplants by intra-oral roentgenologic examination.

A number of investigators have demonstrated varying degrees of differentiation in whole or partial tooth germs which have been grafted into heterotopic positions (1–8) or explanted (5, 9–11). Common sites of transplants have been subcutaneous (1, 2, 3), intramuscular (2, 3), and intracerebral locations (4, 5). Other areas selected for the reception of grafts are the marrow cavity of the tibia (6), the ovary (8) and the chorio-allantoic membrane of the chick (8).

So far as we are aware, the use of the alveolar socket as a site for tooth germ transplants has been reported previously only by Kostecka (3) and by Lapchinsky and Malinovsky (12). Kostecka transplanted the germ of the first permanent mandibular molar of dogs and white mice in place of the corresponding germ on the opposite side of the same animal and found that the transplants were absorbed in from 3½ to 5½ months. The experiments of Lapchinsky and Malinovsky first came to our attention in a newspaper article in 1941 and the only further reference to them which we have been able to find appears in an editorial in the Lancet (12). According to the latter account, these investigators transplanted tooth germs from young puppies into the alveolar sockets of adult dogs. Not only are the fresh transplants described as having grown to full size in their new position, but similar successful results are said to have been obtained if the donor was sacrificed on the day before operation, and its jaws kept overnight in a refrigerator.

The present paper reports some of our findings to date. These strongly suggest that intra-alveolar tooth germ transplants are capable of differentiating and persisting.

[1] Presented at the meeting of the International Association for Dental Research, New York Section, January 29, 1945.

MATERIALS AND METHODS

The cat was selected for this study because the structure (13) and order of development (14) of its dentition are closely similar to those of the primate, and the dentition is completely developed in 9 months (15). *Figs. 1* and *2* are diagrams of the developing maxillary and mandibular dentition of young cats. The dental index of the adult cat is $i\frac{3}{3}$, $c\frac{1}{1}$, $pm\frac{3}{2}$, $m\frac{1}{1}$. The maxilla has 3 premolars on each side of the median line, whereas the mandible has but 2. With the exception of the permanent molars, all of the permanent teeth are preceded by a corresponding number of deciduous teeth. Incisor teeth in this discussion are referred to as 1, 2 and 3 according to their position in relation to the midline, where the numbering is begun.

Fig. 1. Diagram illustrating maxillary dentition of young cat. Heavy outlines, deciduous teeth; cross-hatching, permanent teeth.

Earlier studies by one of us (H. H. S.) have enabled the determination of the approximate age of cats by roentgenologic examination of the dentition (15). The same author has developed a technic for the removal of the intact developing tooth germ (16). Completeness of the removal is attested by the fact that no regeneration follows the operation.

The 14 animals serving as hosts in these experiments ranged in age from $1\frac{1}{2}$ months to 1 year; the donors from $4\frac{1}{2}$ weeks to $5\frac{1}{2}$ months. The following operations were performed:

Group 1. Replacement of right permanent mandibular canine of host by the germ of a similar tooth from a younger animal (8 cases).—A. By right developing permanent mandibular canine (5 cases); B. By left developing permanent man-

dibular canine (2 cases); C. By right developing permanent maxillary canine (1 case).

Group 2. Replacement of host tooth by the germ of a different tooth from another animal (6 cases).—A. Right permanent mandibular canine replaced by the right permanent maxillary incisor of donor animal approximately the same age (4 cases); B. Right permanent mandibular first premolar replaced by left developing permanent mandibular canine of a younger animal (1 case); C. Right permanent mandibular premolars 1 and 2 replaced by right developing permanent maxillary incisors 1, 2, 3 of a younger animal (1 case).

Fig. 2. Diagram illustrating mandibular dentition of young cat. Heavy outlines, deciduous teeth; cross-hatching, permanent teeth.

TECHNIC

The prospective host was anesthetized with ether or intraperitoneal injections of nembutal. (In our experience, 20 to 30 mgm. of nembutal per kilogram of weight of the animal is an effective dosage for kittens.) Following anesthesia, an incision was made through the skin and soft structures overlying the lower border of the mandible of the host, the tissue retracted, and a small window cut through the outer alveolar plate with a surgical bur. The exposed permanent tooth was then removed from its bony socket and the wound protected by sterile gauze. A similar operation was at once performed upon the donor, the developing tooth germ transferred from it into the space previously prepared in the host animal, and the soft tissues and skin sutured. Intra-oral roentgenograms were

taken immediately preceding and following each operation and continued at intervals in the host animal, and in some cases, in the donor also.

In a few cases, in preparing the site for receiving the graft, the germs of adjacent teeth were removed (see observations, case 5).

OBSERVATIONS

Detailed description is given of two cases representing each of the groups described.

Group 1. Transplanted tooth of the same type (canine) as the tooth it replaced, the donor being younger than the host. *Case number 5.* From a cat, about 4 months old, the lower right permanent canine tooth was removed and in its place was transplanted the corresponding tooth germ of a 4½ week old kitten. *Figs. 3a* and *3b*, show roentgenograms of the donor and host mandibles before operation. *Fig. 3c* shows the host mandible immediately after operation, with the grafted canine germ in position in the canine socket of the host. It may be noted that the permanent first and second incisor germs were removed on the operated side. In subsequent illustrations of later stages in this animal, the displaced permanent third incisor germ on the operated side may be seen developing above the crown of the transplant.

In a roentgenogram taken eight days after transplantation (*fig. 3d*) the transplant shows no conspicuous change. However, during the following 2 weeks (*figs. 3e* and *3f*), the enamel organ loses its opacity and becomes vague and shadowy in the roentgenogram, although the papilla remains fairly distinct. Five weeks after operation (*fig. 3g*) the opacity of the crown begins to increase, and this condition progresses in the subsequent 4 weeks (*figs. 3h* and *4a*), during which time the papilla also appears to be differentiating. Accompanying these changes in the transplant, there is increased evidence of the development of the displaced permanent third incisor which overlies the crown of the transplant.

The same area 1 month later (*fig. 4b*; 91 days after operation) shows continued growth of the transplant and of the adjacent permanent third incisor. On this side, the deciduous canine is absent, having been lost 21 days earlier. On the control side, the deciduous canine is just about to be shed.

At the next age shown, 3 weeks later (*fig. 4c*; 112 days after operation), considerable growth seems to have occurred in the crown and root of the transplant. The position of the overlying permanent third incisor suggests a mechanical interference with the growing crown of the transplant. *Fig. 4d* and *4e* (133 and 161 days after operation) emphasize these changes. The root of the transplant resembles closely that of the contralateral developing canine of the host. The deeper position of the transplant is not surprising in view of its small size at the time of the operation, and the relatively large socket into which it had been placed.

Fig. 4f (10 months after operation) shows the loss of the permanent third incisor on the operated side. This tooth had erupted and was detached from its loose connection to the gingiva. The root of the transplant is completely formed,

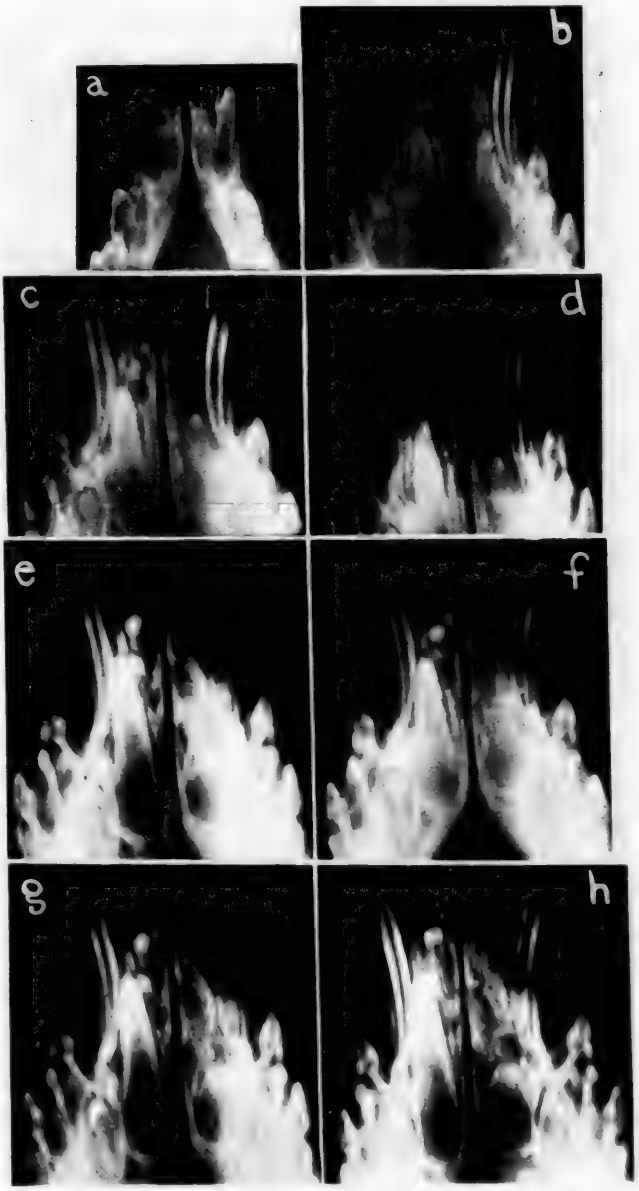

Fig. 3. Series of roentgenograms showing progress of right permanent mandibular canine of 4½ week old kitten transplanted in place of corresponding tooth of 4 months old cat (case no. 5). *a*, donor mandible before operation; *b*, host mandible before operation; *c*, host mandible immediately after operation; *d h*, host mandible at stated periods after operation: *d*, 8 days; *e*, 14 days; *f*, 21 days; *g*, 35 days; *h*, 49 days.

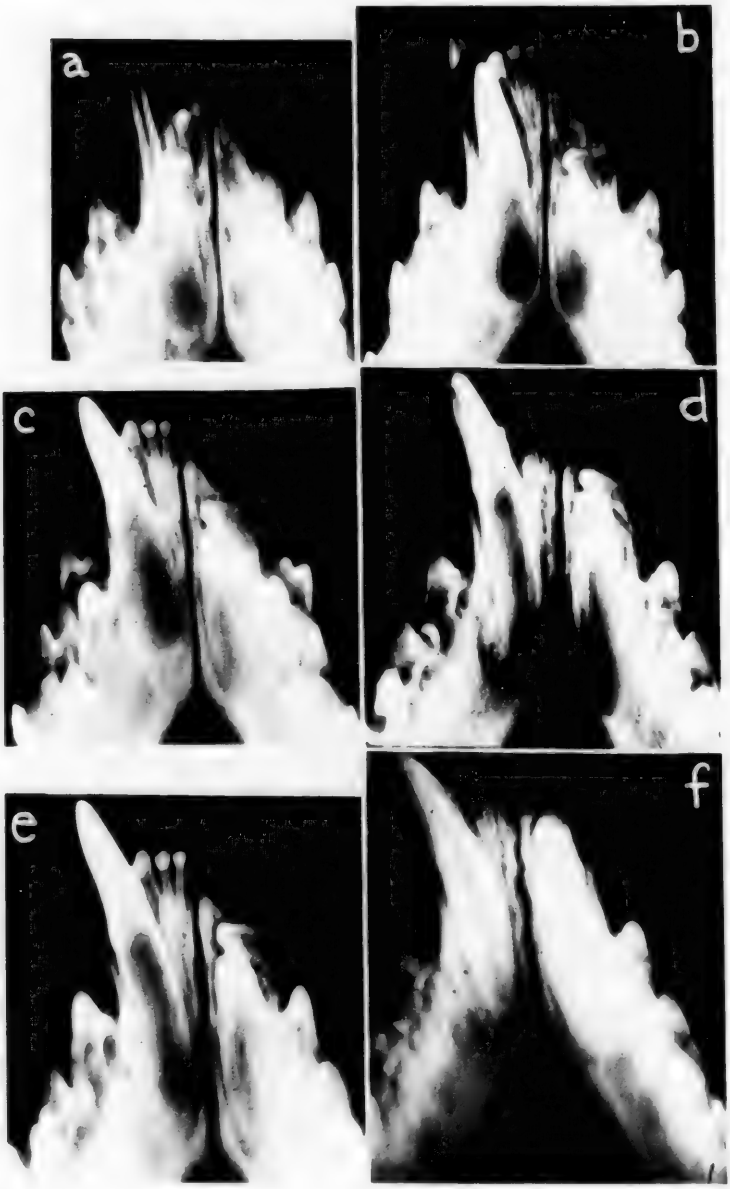

Fig. 4. Case no. 5, continued from *fig. 3*, showing progress of transplanted canine in host mandible at stated periods after operation: *a*, 63 days; *b*, 91 days; *c*, 112 days; *d*, 133 days; *e*, 161 days; *f*, 304 days.

its apex situated deep in the mandible. At the apex of the incompletely developed crown, resorption of the overlying alveolar bone is evident, suggesting that normal eruption is in progress.

This animal lived for approximately 2 years after operation. The transplanted canine persisted throughout that period without evidence of resorption but failed to erupt into the oral cavity. The extent of its development and eruption may perhaps be explained first, by the juxtaposition of the displaced permanent third incisor of the host, which appeared to impede the formation of the crown of the transplant; secondly, by the fact that the graft occupied a deeper position than normal, in the mandible of the host.

The other 7 operations of this group survived, or were kept, for 1, 10, 16, 20, 41, 160 days, and over 1 year, respectively. Except for the 1 day survival, all of these showed an initial decrease in opacity of the enamel organ occurring in the second week after operation. Those surviving beyond the fifth week showed a gradual increase in opacity and a consequent clearer definition of the enamel portion of the graft. This was observed even in 2 cases (41 and 160 days) in which the later development of the graft indicated that it had been injured at the time of the operation; and in another animal which was kept for more than 1 year, in which the graft became dislodged from the alveolus and continued its development into a hard nodule beneath the skin along the border of the mandible.

Group 2. Transplanted tooth of a different type from tooth it replaced. *Case number 11.* The right mandibular canine tooth of this animal, aged 6 months (*fig. 5b*) was removed surgically and replaced by an almost completely developed right maxillary permanent third incisor of another animal aged 5½ months (*fig. 5a*), approximately the same age as the host. The crown and a portion of the root of the maxillary incisor were well developed at the time of transfer to the mandible of the host. The third permanent incisor of the left side served as a control.

Fig. 5c shows the maxilla of the donor immediately after removal of the tooth serving as the transplant. It will be observed that the deciduous canines are present at this stage, only the deciduous incisors having been shed. *Fig. 5d* shows the mandible of the host immediately after the transplant had been placed in the socket of the removed permanent canine. The crown and partially formed root of the grafted incisor may be seen clearly in this view. It is noteworthy that the transplanted maxillary third incisor is larger than the mandibular incisors of the host. On the unoperated side of the host, the developing permanent canine may be seen. In the mandible, the deciduous incisors and canines have been shed; only permanent teeth are present. *Figs. 5e* and *5f* show similar views of the maxilla of the donor and the mandible of the host, 6 days after operation.

In a roentgenogram of the donor maxilla 22 days after operation (*fig. 5g*) the control incisor may be seen clearly. Its root has not as yet completely formed. Bony regeneration is occurring in the socket of the removed third incisor of the opposite side. At the same stage, the host animal (*fig. 5h*) shows bony regeneration occurring also in the area of the transplant, and the tooth is seen establishing itself. The root portion is slightly less opaque than the corresponding area of the control tooth in the donor. Thirty-six days after operation, the root of the control tooth may be seen to be almost completely formed (*fig. 5i*). In

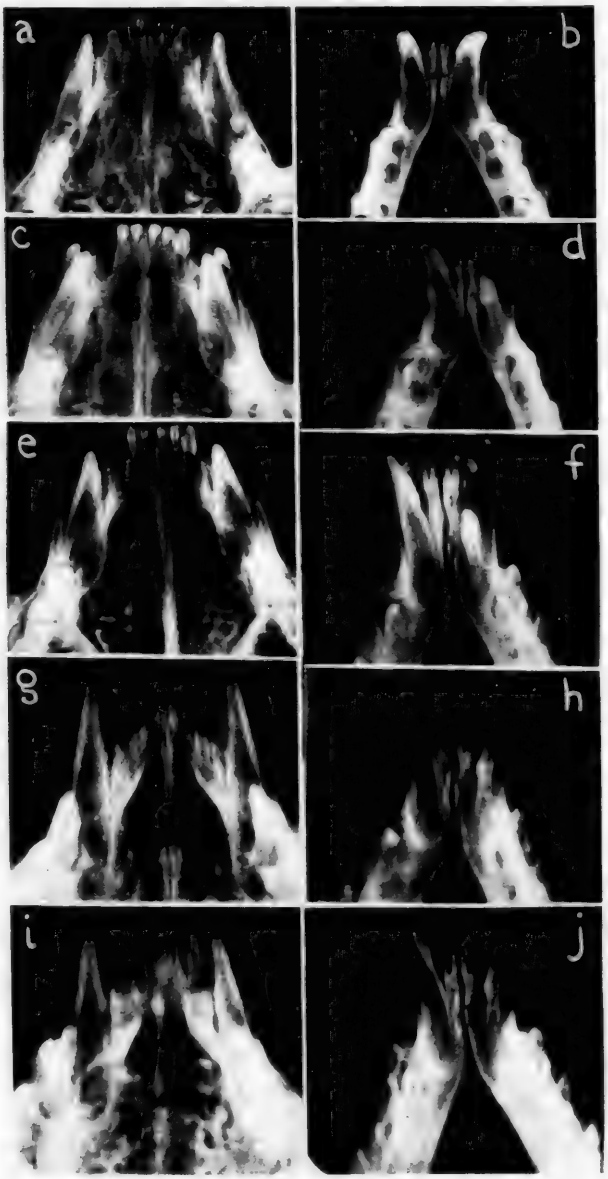

Fig. 5. Series of roentgenograms showing progress of right permanent maxillary third incisor of 5½ month old cat transplanted in place of right permanent mandibular canine of 6 month old cat (case no. 11). *a*, donor maxilla before operation; *b*, host mandible before operation; *c*, *e*, *g* and *i*, donor maxilla; and *d*, *f*, *h* and *j*, host mandible, at stated periods after operation: *e* and *f*, 6 days; *g* and *h*, 22 days; *i* and *j*, 36 days.

fig. 5j, the root of the transplant in the mandible of the host appears to be in a similar state of development.

The donor in this experiment was the reciprocal of case number 11, receiving from the latter a right permanent maxillary third incisor in place of the right mandibular permanent canine. Roentgenograms taken at corresponding intervals after operation showed similar progress in the transplant.

These 2 animals lived 52 days longer, and formed part of a demonstration at meetings of the American Association of Anatomists in April, 1942 (17). At that time (85 days after operation) the transplanted incisors of both animals were beginning to erupt into the oral cavity and could be seen and palpated.

Two more operations of this type were performed with animals of 3½ and 4 months of age serving as reciprocal donors and hosts. These survived 14 and 19 days following operation. In the case of another animal, the right permanent mandibular premolar was replaced by a mandibular canine from the left side of the jaw. In this case, 7 days after operation, the transplant showed an unaltered appearance in the roentgenogram.

The last case in this group involved the transplantation of maxillary right permanent incisors 1, 2 and 3 in place of mandibular right premolars 1 and 2. The transplants appeared opaque in successive postoperative roentgenologic examinations. However, 41 days after operation, 1 of the transplants could no longer be seen in the roentgenogram, and 24 days after that time, a second could not be detected. Since there had been no prior indication of resorption, it is believed that the transplants remained healthy and underwent differentiation, but failed to become attached within the large socket in which they were placed; hence they were shifted buccally and lost. The remaining transplant showed development of the root and presented an opaque and typical appearance up to 75 days after operation. During the next 2 weeks it began to disappear in the soft tissues buccal to the alveolar plate, and only the tip of the crown was visible in the latest roentgenogram taken before death, 93 days after operation.

We are continuing our experiments and accumulating material for essential histological study in order to determine the extent of development and differentiation undergone by intra-alveolar tooth germ transplants such as have been described in this study.

SUMMARY

1. Developing permanent canine or incisor teeth of kittens were transplanted in place of permanent canine or premolar teeth of older animals and, in a few cases, of young animals approximately the same age as the donors.

2. Intra-oral roentgenograms taken at frequent intervals after operation indicated that the transplant, if intact, became established in the alveolar socket of the host, and continued its development. Injured transplants underwent gradual resorption.

3. A decrease in radiopacity during the first 3 to 5 weeks after operation was evident in transplanted canine germs, but inconspicuous in transplanted incisor germs whose crowns were completely formed at the time of operation. Transplants persisting beyond 5 weeks showed a gradual reappearance of opacity.

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THE EFFECTS OF VARIOUS IONS ON ENAMEL SOLUBILITY[1]

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Several reports in the literature indicate that the solubility of enamel in acids can be changed by the action of various ions. Miller (1) using silver nitrate was unable to show retardation in enamel decalcification. The research work of Rickert (2) contradicted this and led him to believe that increased resistance to decalcification of silver nitrate-treated enamel was due to the clogging of tooth substance with reduced silver which prevented the penetration of acids. Hill and Arnold (3) studied the effects of silver nitrate on powdered enamel and found that after treatment, the solubility of enamel was significantly reduced. Difference in enamel solubility in acetate and maleate buffers was attributed by Benedict and Kanthak (4) to the formation of calcium maleate which acted as a protective coating over the enamel particles. Volker (5) and Bibby (6) investigated the effect of fluorides on enamel and concluded that the fluorine ion produced marked reduction in solubility. Because little or no consideration has been given to the effects of other ions on the solubility of enamel, it seemed to us worthwhile to investigate the matter.

The choice of the ions used in the experiments was determined by a consideration of the mechanisms whereby the phenomenon of reduced solubility might occur. In order to better understand these, an examination of the chemical nature of enamel is necessary. This will be limited to facts relevant to the adequate understanding of certain assumptions which are made in this paper.

Tooth enamel is a crystalline substance showing a characteristic x-ray diffraction pattern. Moreover, this pattern is practically identical with that given by bone, dentin and certain synthetic or natural inorganic materials, such as fluorapatite. On the basis of these x-ray findings these substances have been included in the general class of apatites. It should be remembered that this apatite classification refers only to a particular atomic pattern or arrangement in the crystal lattice and not to any constancy of chemical composition. For instance, McConnell (7, 8) has pointed out that a large number of substitutions are possible in the general apatite pattern. If $X_{10}(ZO_4)_6 \cdot (F_2OH_2Cl_2)$ represents the general formula for the apatites, then X may be calcium, lead, manganese, potassium, strontium and cesium or in lesser amounts yttrium, lanthanum, titanium, iron, aluminum and magnesium; Z may be chromium, phosphorous, arsenic, vanadium, silicon, and carbon. Further evidence of the complex and variable nature of the apatites is obtained from the work of Eisenberger, Lehrman and Turner (9). In their critical review of the literature on the system

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P_2O_5-CaO-H_2O, to which enamel belongs, they have stated ". . . it is contended that at least for hydroxyapatite, a substance with small whole number ratios of atomic species does not exist but rather a crystal lattice common to a continuous series of solid solutions." "It follows from this that tricalcium phosphate and hydroxyapatite do not exist in aqueous systems as unique stoichiometric compounds." Essentially then a definite chemical formula representing a true molecular species cannot be ascribed to hydroxyapatite. What may be said to actually exist is a variety of chemical combinations among substances capable of being ultimately expressed in ratios of P_2O_5-CaO-H_2O. For enamel, analysis shows that the ratios of the major constituents can be best expressed by the formula $3Ca_3(PO_4)_2 \cdot Ca(OH)_2$. This formula is essentially an expression of convenience and does not necessarily indicate the true state of combination of the elements present. The possibility of substituting for P_2O_5 and CaO varying quantities of other oxides and compounds which are adequately adapted to become part of the lattice is evident. According to Bales (10) the substance of teeth and bones probably presents a considerable lack of chemical homogeneity, presumably resulting from such substitutions in the crystal lattice.

Evidence that other elements than those identified in the typical apatite formula occur in enamel has been shown in both chemical and spectrographic analyses. These have revealed the presence of a variety of substances including carbon dioxide, nitrogen, magnesium, zinc and others (11, 12, 13). Of these adventitious constituents, CO_2 has been reported in the largest amounts, but significantly different quantities have been reported by various investigators (14, 15). The manner in which the CO_2 is bound in the apatite lattice has been disputed (16, 17, 18, 19, 20, 21). While some claim that it is part of the fundamental apatite pattern, others hold that it is not part of the apatite molecule but exists in the form of the carbonates of magnesium and calcium. Other ions such as magnesium, calcium and sodium, which are found in enamel are considered as integral parts of the main apatite lattice (22, 8, 23).

Since the evidence concerning hydroxyapatite is directly applicable to enamel, it is evident from the above that the enamel substance allows for very interesting possibilities in the way of chemical reaction. Just as $Ca_3(PO_4)_2$ will react with solution of CaF_2 (24, 25) to form by addition reaction the relatively insoluble fluorapatite and just as calcium hydroxyapatite will react with soluble fluorides to form by exchange reaction fluoroapatite $3Ca_3(PO_4) \cdot CaF_2$ (26), so the possibility exists for similar addition or exchange reactions between the constituent ions and molecules of enamel and other ions, such as Pb, oxalate, SO_4, etc. Such reactions might produce compounds less soluble than the original, such as a highly insoluble lead hydroxyapatite, the existence of which has already been shown (27). For our experiments we chose such ions as sulphate, oxalate, fluoride, lead and strontium because of their tendency to form compounds of low solubility and the likelihood that combination between such ions and enamel would produce less soluble compounds than those which may be considered as normally existing in enamel itself. Such new compounds could be of the nature of $Pb_3(PO_4)_2$, $Sr_3(PO_4)_2$, CaC_2O_4 all of which are known to be less soluble than

$Ca_3(PO_4)_2$ or $Ca(OH)_2$ (28). New compounds of this sort whether they are precipitated on the surface of the enamel particles or incorporated, as a result of a chemical reaction in the crystal lattice, might produce interesting effects on the solubility of enamel. The experiments to be reported were undertaken to test the application of the general hypothesis outlined above to the problem of modifying the solubility of enamel.

EXPERIMENTAL

The enamel used in our experiments was prepared according to the method of Manly and Hodge(29). It was powdered to pass through a 100 mesh screen and stored in a desiccator. Reagent solutions were prepared in strengths of 1/500 using soluble compounds of the ions to be studied. Because previous tests had shown more marked effects on enamel solubility when acid solutions were used, the diluent generally used in making all solutions was sodium acetate—acetic

TABLE I

Average results of solubility tests

REAGENT SOLUTION 1/1000 CONCENTRATION	pH OF REAGENT SOLUTION	LOSS OR GAIN IN WT. IN MGS. AFTER TREATMENT WITH REAGENT	TOTAL LOSS IN WT. IN MGS. AFTER TREATMENT WITH REAGENT AND ACID
*PbF_2	3.90	+3.7	4.9
$Pb(NO_3)_2$	3.98	+1.50	9.4
$Na_2C_2O_4$	4.08	−3.5	20.3
$CuSO_4$	3.91	−3.6	24.3
NaF	4.08	−4.3	17.0
$Zn(NO_3)_2$	3.95	−5.2	23.8
$AgNO_3$	3.95	−8.7	30.8
$SrCl_2$	4.03	−9.6	28.3
Na_2SO_4	3.96	−11.6	32.9
Acetate buffer	4.0	−10.7	32.7

* Concentration of the lead fluoride solutions were 0.6 parts per 1000.

acid buffer of 0.2 M strength and having a pH of 4.02. All further dilutions of the reagent ions were made with this buffer. Because of the insolubility of the lead fluoride similar concentrations could not be used, but instead enough of the compound was weighed out to give an approximately saturated solution having a concentration of 0.6 parts PbF_2 per 1000. The solution used for decalcification was the pH 4, 0.2 M sodium acetate-acetic acid buffer used for making the reagent solutions. The pH values of the reagent solutions were determined on the glass electrode.

In making the solubility tests 5 c.c. of one of the reagent solutions was added to 100 mg. of the powdered enamel contained in tared Royal Berliner type filtering crucibles. The mixture was then stirred for 20 minutes by a motor driven, glass rod, after which any enamel adhering to the rod was washed into the crucible with 1–2 c.c. of water. The entire contents of the crucible were then filtered under suction and washed 3 times with distilled water. Thereafter,

20 c.c. of the pH 4 sodium acetate-acetic acid buffer were added. This was stirred, filtered and washed as previously described, and dried for 1 hour at 110° C. After cooling, the crucible was placed in a desiccator for 24 hours and then weighed. The difference between the initial and final weight of the crucible represented the loss in enamel substance. Each run was made in duplicate.

To determine whether any changes in weight were brought about by the action of reagent solutions, duplicate runs were made as above, but drying and weighing was done after the treatment with the reagent without exposure to the decalcifying buffer.

The results obtained are given in Table I. These values represent averages of 2 or more determinations differing by not more than 10%. The table also includes control values obtained by using the acetate buffer alone. Some tests using non-acidulated reagents and lactic and citric acid buffers gave definite but less marked solubility reductions.

DISCUSSION

The most interesting result of these experiments is the finding that the lead ion, alone or in combination with the fluoride ion, is very effective in reducing the solubility of the enamel. With the exception of the oxalate ion which is comparable to fluoride ion, the other ions tested are less effective than fluoride in reducing enamel solubility under the conditions of the experiment.

Since fluoride alone is much less effective than lead or a combination of lead and fluoride, the reduction of solubility must be considered as being due either to a reaction with the lead ion or the lead fluoride molecule. The increase in weight of the enamel after treatment with lead fluoride and lead nitrate (Column A, Table I) casts some light on the nature of the reaction but still leaves four possibilities:

(1) Adsorption of the lead ions on the surface of the enamel particles,
(2) Incorporation of the lead ions in the enamel substance in place of calcium ions,
(3) Combination of the lead ions with phosphate or carbonate ions etc., derived from the enamel, to form insoluble lead compounds which precipitated as a protective layer over the enamel particles,
(4) An addition reaction between PbF_2 and $Ca_3(PO_4)_2$ similar to that occurring between CaF_2 and $Ca_3(PO_4)_2$.

The first supposition involves no chemical reaction and the reduction in solubility could be explained in 2 ways: (a) the presence of lead ions may prevent acid action by virtue of a protective effect; or (b) a salt effect whereby the presence of lead ions already in solution inhibit any chemical process which would tend to increase the ionic concentration of the solution.

The second and third possibilities are based on a chemical substitution reaction. In the former case there is an isomorphous type of substitution of Pb for Ca ions. In the latter a certain amount of solution of the enamel with liberation of phosphate and CO_3 ions and others is induced. These ions, in turn combining with lead ions, form insoluble substances which precipitate and form a coating

on the enamel particles. This coating prevents ready access of acid and thus slows solution.

Basically both the above mechanisms of solubility reduction are the same, depending upon the production of insoluble substances more resistant to acid action than the enamel itself. Moreover, the compounds formed, whether they are part of the crystal lattice or not, may be considered identical since lead ion merely takes the place of the calcium. If it is assumed that each lead ion takes the place of one calcium ion, then, in order to account for the 3.7 mg. increase in weight of the enamel after treatment with PbF_2, 4.6 mgs. of lead are required in the reaction. This amount equals 0.000022 mols of lead ion which if converted to calcium equivalents represents 1 mg. of calcium ion. This is the amount of calcium that would go into solution. In terms of enamel substance, considered as hydroxyapatite, the amount is small and does not make it possible to account for the decrease in solubility as being the result of the greater weight of the lead ion giving false values for the enamel weight.

That this effect does not take place to the same extent when lead ion is used in the form of the nitrate, brings up the question of the rôle of the fluoride ion in the reaction. On one hand the possibility exists that the fluoride ion replaces the hydroxyl ion of enamel with the formation of fluorapatite (in addition to which Ca is substituted by lead). This fluoride reaction results in the production of a compound more resistant to the action of the acid used than those compounds produced when lead acts alone. The type of reaction taking place could be that suggested in the last supposition. When $Ca_3(PO_4)_2$ reacts with CaF_2 the product formed is considered to be $3Ca_3(PO_4)_2 \cdot CaF_2$. A similar reaction between PbF_2 and the calcium and phosphate ion derived from enamel is entirely possible. This product, either by precipitation on the surface of the particles, or by incorporation through a chemical reaction in the crystal lattice, renders the enamel substance less soluble to the acetate buffer.

It is not possible to say which process actually takes place. Perhaps it is best to consider each one as contributing to the whole effect. However, this fact is evident, that the fluoride and lead ions acting together produce greater solubility reduction than if employed individually.

If similar mechanisms of reaction are considered operative for the other ions, then it must be concluded that the reaction products formed are not as resistant to the action of the acid used as those formed by lead alone or in combination with fluoride.

On the basis of the results obtained it might seem justifiable to state that since topical applications of sodium fluoride have given promise in the control of dental decay, it seems logical that the use of lead fluorides might be even more effective. The demonstration that the solubility of the enamel is affected by ionic reactions suggests that they would influence the composition of dentin and bone and modify their liability to decalcification and resorption. Therefore, it seems likely that an application and expansion of the sort of study we have reported here might contribute much to our understanding of a variety of bone disorders.

SUMMARY

The possible influences of various ions on the solubility of enamel has been discussed and experiments carried out to test these effects.

Lead ion alone or in combination with fluoride has been found to be very effective in reducing enamel solubility.

Possible reaction mechanisms to explain this phenomenon of reduction in solubility have been postulated.

The possibility of clinical use of lead fluoride in the control of dental decay has been suggested.

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THE RELATIONSHIP BETWEEN MICROSCOPIC ENAMEL DEFECTS AND INFANTILE DEBILITIES[1, 2]

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Defective structure of the teeth, particularly what is known as hypoplasia, has been the subject of observation and study by dentists from earliest times. Various types of enamel defects (gross hypoplasia) have been recognized and various explanations have been offered regarding their cause. The exanthematous diseases of childhood have come under particular suspicion and some writers have gone so far as to attribute different types of hypoplasia to certain specific exanthematous, or other, diseases and to those diseases alone. While no credence can now be given to this idea of specific causation the observations which gave rise to the concept were important because they indicated that sickness in early life adversely affected the structure of the teeth.

Two series of studies have advanced our knowledge of the influence of systemic factors on enamel formation. In 1 of these Mellanby (1) has shown that a deficiency of Vitamin D during the period of tooth formation gives rise to deficient tooth formation which manifests itself as varying degrees of irregularity of the enamel surface according to the severity of the deficiency. The other series of studies began with Berten (2) who offered evidence to show that transitory disturbances in body metabolism reflected themselves in the microscopic structure of the teeth. This work has recently been confirmed and expanded by Massler, Schour and Poncher (3) and by Sarnat and Schour (4) who drew particular attention to the "neonatal line", a defect in the enamel which they suggest is the result of metabolic disturbances in the infant brought about by birth and adjustment to extra-uterine life. It is not clear from the published reports which types of systemic affections are most conducive to defective enamel structure or whether such structural defects invariably follow general metabolic upsets.

The study we are recording was prompted by the availability of a group of teeth collected from children with detailed life-long medical histories which seemed to present an opportunity for checking the earlier observations and perhaps expanding our knowledge on the effects of systemic debilities on the structure of the enamel.

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MATERIALS AND METHODS

The teeth used in this study were extracted in the dental clinic of the Boston Dispensary from children in the 'Preventive Group'. These children had been under the care of Dr. Francis C. McDonald, Assistant Physician-in-chief, of the Boston Floating Hospital, for periods up to seven years. Complete medical histories, including prenatal and dietary records, were available for all patients. For study purposes ground sections of the teeth were prepared on a rotary grinding stone. The preparation of satisfactory sections was made difficult by reason of the decayed state of many of the teeth and because the teeth had become completely dry and, therefore, extremely subject to fracture during the grinding process. Nevertheless, a total of 95 satisfactory ground sections were prepared from one or more teeth of 34 individuals. These were first examined under a binocular microscope giving the magnification of 20 diameters. When indicated, further study was made under a low power (100 ×) regular microscope. Because of a variety of types of enamel defects noted in our sections, it was impossible to attempt to correlate all of them with the systemic background. Therefore, our study was limited to a consideration of linear defects which could be traced circumferentially around at least two-thirds of the entire enamel. The calcification tables of Schour and Massler (5) were used to estimate the approximate times of any disturbance in enamel formation. The estimation of the times of such disturbances was aided by identifying the position of the neo-natal line which seldom presented a problem in this group of teeth. The estimated time of occurrence of all such defects was recorded for all sections before any of the case histories were read. Obviously, the arbitrary choice of a standard for inclusion of defects may be open to some criticism because of the omission from our records of many definite but minor defects. However, since the same standards were used throughout and all decisions were made by a single examiner in ignorance of the case histories, the results cannot have been influenced in any particular direction.

After a study of all the sections from all the cases was completed, the case histories were read and summarized for systemic disturbances. Then the periods of occurrence of nutritional deficiency, illnesses, disturbances in metabolism, etc., were compared for correlations with the estimated times of formation of the structural defects recorded from the histological study. The results were then grouped according to the nature of the correlations.

RESULTS

A summary of the cases in which positive correlation was found to exist between the period of calcification defect and a known systemic disturbance is given with certain relevant data in Table I. In Table II the cases showing marked disturbances in enamel calcification without any recorded systemic involvements are presented. Table III lists 2 cases in which conditions which caused enamel defects in other patients failed to produce structural changes in the teeth.

It is interesting to note that in those instances in which sections of more than 1 tooth from a patient were examined the structural defects appeared in correspond-

TABLE I

Positive Correlations —Defect and Cause

CASE #	TOOTH STUDIED	OBSERVED ENAMEL DEFECT			CORRESPONDING SYSTEMIC INVOLVEMENT	
		Number of Bands Present	Estimated Age of Onset	Estimated Duration	Nature	Time
1	$\overline{E}$	1	10 mo.	1 wk.	Measles	1 yr.
2	$\underline{6} + \underline{6}$	3	7 mo. 1½ yrs. 2½ yrs.	1 wk. 1 mo. 1 mo.	Chicken pox Pneumonia Pneumonia	6 mo. 1 yr. 2 yr.
3	$\underline{D}$	1	3 mo.		Pertussis	3 mo.
4	$\underline{B}$	Pigmentation of all enamel	4 m.i.u.–2 mo.	7 mo.	Vit. D def.	4 m.i.u.–2 mo.
5	$\underline{A}$	PreN and PostN E pigmented	4 m.i.u.–2 mo.	7 mo.	Vit. D def.	4 m.i.u.–2 mo.
8	$\underline{C}$, $\underline{D}$	1	5 mo.	3 mo. (all of PostN enamel)	Eczema of scalp—Possible vit. A def. and vit. D def.	5–17 mo.
9	$\underline{D}$, $\overline{D}$	1	11 mo.	1 wk.	Chicken pox	1 yr.
10	$\underline{8}$	Granular, poorly formed postN enamel	Birth to 3 mo.	3 mo.	Grossly deficient diet in vit. A, C, D, and Ca, P, & Fe.	
13	$\overline{A}$	PreN E poorly calcified	4 m.i.u.–birth	5 mo.	Vomiting of mother, possible toxemia of preg.—Deficiency in vit. A, C, & D. Def. of Ca, P, & Fe.	2nd & 3rd trimester of preg.
14	$\overline{E}$, $\underline{E}$, $\underline{E}$, $\underline{6}$, $\overline{6}$	1 wide band	Birth–2½ yrs.	2½ yrs.	Vit. D. def.	2½ yrs.
16	$\overline{D}$, $\overline{E}$	1	3–6 mo.	3 mo.	Diarrhea, cold, fever, purulent nasal discharge, bilateral otitis media and purulent upper respiratory infection	3 mo.

TABLE I—*Continued*

CASE #	TOOTH STUDIED	OBSERVED ENAMEL DEFECT			CORRESPONDING SYSTEMIC INVOLVEMENT	
		Number of Bands Present	Estimated Age of Onset	Estimated Duration	Nature	Time
17	$\underline{\lfloor C}$	I	6 mo.	3–4 wks.	Inguinal hernia, intestinal obstruction	5½ mo.
18	$\overline{\lceil D}, \overline{\lceil E}$	All E poorly formed	Birth	2½ yrs.	Vit. C & D def.	2½ yrs.
19	$\underline{\lfloor E}$	1 broad band	Birth	1 yr.	Vit. C & D def.	1 yr.
20	$\overline{\lceil D}$	I	1–2 mo.	1 mo.	Vit. D def.	2 mo.
21	$\overline{\lceil 6}$	1 broad band	1½ yrs.	1½ yrs.	Vit. D def.	3 yrs.
24	$\underline{\lfloor A}, \underline{\lfloor C}$	All PreN & PostN enamel pigmented	4 m.i.u.	14 mo.	Vit. D def.	PreN and PostN
25	$\overline{\lceil D}, \overline{\lceil E}, \overline{E\rceil}$	Broad NeoN line	6 m.i.u.	9 mo.	Toxemia of preg.—Vit. D def.	3rd Trimester of preg.
26	$\overline{E\rceil}, \overline{\lceil D}$	All of PreN and PostN enamel	6 m.i.u.	14 mo.	Vit. D def.	PreN and PostN E
28	$\overline{E\rceil}$	All PreN and PostN E is pigmented	6 m.i.u.	14 mo.	Vit. D def.	PostN
29	$\overline{D\rceil}$	Widened NeoN line —Heavily pigmented	6 m.i.u.	3 mo.	Cont. vomiting of mother due to stomach ulcers. Pyloric stemosis and op. on infant	3rd Trimester of preg.
30	$\underline{D\rceil}, \underline{\lfloor D}, \overline{D\rceil}, \underline{\lfloor D}, \underline{\lfloor E}$	Series of bands and pigmented E	Birth	11 mo.	Def. in Ca, P and vit. D	PostN
31	$\overline{\lceil D}$	I	2–4 mo.	2 mo.	Stomach disorde —cont. vomiting	4–6 mo.
40	$\underline{\lfloor E}, \overline{\lceil D}, \overline{\lceil E}$	1	8 mo.	1 mo.	Pertussis	11 mo.

Abbreviations used in tables:

E..... enamel
m.i.u..... months in utero
NeoN..... neonatal
PreN..... prenatal
PostN..... postnatal
Preg..... pregnancy

ing places in all teeth. In general, tooth formation was very poor as judged by accepted histological standards. The enamel was fairly generally pigmented and granular; and the dentin showed interglobular spaces. The state of the teeth indicated that a generally low nutritional state existed during the period of their formation.

Twenty-four of the 34 cases studied are included in Table I, indicating that in about 75% of them there is an apparent relationship between the time of the

TABLE II

Negative Correlation—Defect Without Cause

CASE #	TOOTH STUDIED	NO. OF BANDS PRESENT	ESTIMATED AGE OF ONSET	ESTIMATED DURATION	DESCRIPTION OF DEFECT
6	$\underline{E}$, $\overline{D}$	1	6 m.i.u.	13 mo.	E is pigmented—series of five lines just outside neonatal line
7	$\overline{D}$	1	2 m.a.b.	1 wk.	Fine linear defect
11	$\overline{D}$	5	10 m.a.b.	10 mo.	Linear defect (might be infancy ring) series of uncorrelated linear markings between neonatal line and infancy ring. Possible postnatal Ca & P deficiency)
22	$\underline{D}$	1	2 mo.	4 mo.	Outer ⅓ of E is pigmented
23	$\underline{E}$	1	5 mo.	5 mo.	Pigmented zone-darkened line on outer border (possible infancy ring)
43	$\overline{D}$	1	3 mo.	1 wk.	Fine linear defect.
26	$\overline{E}$, $\underline{D}$	1	5 mo.	1 mo.	Linear defects (possible accentuation of Vit. D def.)
28	$\overline{E}$	1	9 mo.	1 mo.	Broad band near surface

TABLE III

Cause Without Defect

CASE #	TOOTH STUDIED	TIME OF SYSTEMIC INVOLVEMENT	DISEASE
44	$\underline{B}$	3 mo.	Eczema of scalp—unknown etiology—1 week duration. Also vit. D deficiency
45	$\underline{A}$, $\underline{B}$	PreN & PostN	Vit. D deficiency

systemic disturbances and the expected locations of the deficiencies in enamel calcification. Defective tooth formation was most commonly associated with single or multiple nutritional deficiencies. In this series of 24 cases the nutritional and clinical records of 14 revealed Vitamin D deficiencies; 4 deficiencies of Vitamin C, 4 of Vitamin A, and 3 of calcium or phosphorus. These occurred singly or in combination and sometimes in association with other diseases. The enamel of the patients with nutritional deficiencies was pigmented and granular

or beaded in appearance which, in view of Mellanby's (1) findings, suggests that Vitamin D deficiency was the predominant cause of the imperfect enamel structure.

A few of the cases seem sufficiently interesting to justify a more detailed presentation than can be given in tabular form.

Case No. 2 showed 3 distinct rings, 1 at an estimated 7 months after birth, 1 at 1½ years after birth, and 1 at 2¼ years after birth. The patient's history recorded chicken pox at 6 months, pneumonia at 1 year and pneumonia at 2 years. While there is a slight discrepancy between the estimated time of the disturbances as arrived at by analysis of the tooth sections and that given in the case history, these variations are within the range of individual variations in the times of tooth calcification and completely within the expected accuracy of the method. Both sections, 1 of the upper right and 1 of the upper left first permanent molar, showed rings in the same positions.

Case No. 8. An eczema of the scalp appeared to be the exciting cause of defective tooth development in this case. However, since the case history also revealed an associated deficiency in Vitamins A and D, the scalp eczema, of itself, may not have produced the tooth defects. It is possible that the vitamin intake was at a sufficiently low level that it could neither maintain normal calcification when the scalp disease supervened, nor reestablish it immediately after the clinical signs of the eczema disappeared.

Case No. 16 presented a variety of systemic factors which probably contributed to abnormal dental development. The patient had a "summer cold" at 3 months of age which was complicated by diarrhea and a gastric disturbance and later by a febrile upper respiratory infection, a purulent nasal discharge, and bilateral otitis media. These illnesses lasted 3 months. This period coincides exactly with the estimated time and duration of the disturbance observed in the calcification pattern.

Case No. 17 showed an enamel defect at approximately 6 months after birth. The case history recorded an inguinal hernia and intestinal obstruction at 5½ months of age. The patient was successfully treated but the disturbance apparently left a permanent record in the enamel.

Case No. 31 presented a broad defect which appeared to be caused at from 2 to 4 months of age. Upon examination of the case history, it was found that at 4 months of age the infant had a stomach disorder of unknown etiology. There was continuous vomiting and no food could be taken. Intravenous methods of feeding were employed during the hospitalization period of 2 months. The disorder disappeared and there was no subsequent recurrence.

In the 8 cases listed in Table II noticeable defects were observed which could not be matched with any related systemic ailment. It is possible that some of the defects noted were prominent developmental rings of the sort described by Massler, Schour and Poncher (3). According to these authors, these so-called developmental rings are really expressions of metabolic variations incident to the growth of the developing infant. Thus, while such rings cannot be considered as pathological, they are expressions of significant changes in cellular metabolism

which, however, do not necessarily manifest themselves clinically. Nevertheless, they may be sufficiently important to influence the sensitive process of amelogenesis to cause permanent records in the enamel.

The teeth from the 2 cases recorded in Table III failed to show defects of sufficient prominence to be recorded in our histological study, although their histories revealed a Vitamin D deficiency in both, which in 1 case was complicated by eczema of the scalp, disabilities which had produced definite enamel defects in other children.

DISCUSSION

The distribution of our cases between those which showed correlation, between the occurrence of the enamel defects and systemic diseases (Table I), those which showed enamel defects but no history of illness (Table II), and those which showed no defects where there was a history of systemic disturbances (Table III), seems to indicate rather clearly that, on the basis of the standards used in this study, there is a more or less definite correlation between the occurrence of formative defects in the enamel and contemporaneous systemic ailments. In approximately 70% of the cases with distinct defects of tooth calcification, there were definite records of systemic disturbances at times corresponding to that of the defective enamel formation. In this respect then, we can confirm in general terms Sarnat & Schour's (4) hypothesis that the pattern of enamel structure offers a permanent record of deleterious influences acting on the enamel organ during the period of amelogenesis. It is possible that in our series of cases these records were made less clear and more difficult to interpret as a result of the generally poor enamel formation, which was associated with the rather poor state of nutrition and health in our subjects.

Of the systemic disturbances reflected in enamel pattern nutritional deficiencies were by far the most common. Correlations with chicken pox, common measles, whooping cough, and pneumonia appeared in only 1 case each. This predominance of nutritional deficiencies offers support to Mellanby's claim that enamel hypoplasia is commonly caused by Vitamin D deficiencies. Obviously, compared with nutritional deficiencies, the exanthemata play only a minor part in the causation of enamel defects and contrary to common opinion are not a major cause. It is even possible that the 2 correlations we observed may not have been found had the patients had a more favorable standard of nutrition. The demonstration (6) that experimentally produced fevers in animals manifested changes only in the dentin and especially under unfavorable nutritional conditions offers support to this idea, although the experimental fevers did not involve toxins such as would be encountered in the exanthemata.

It is uncertain what these enamel defects mean in terms of metabolic dysfunction. This is so, not only because of the variety of facts that influence enamel formation, but also because of the uncertainty regarding the actual mechanism of amelogenesis. It seems apparent, however, that amelogenesis is a delicate process which can be disturbed either by deterioration of the ameloblasts themselves or by deficiencies in the supply of the materials from which the ameloblasts

elaborate their calcific secretion. Systemic disturbances can, of course, directly or indirectly produce both of these results. The total impression is that optional enamel formation permits only minor variations in body metabolism; wider variations will apparently be reflected permanently in the structure of the enamel.

SUMMARY

Ground sections of 95 teeth from 34 patients with detailed medical histories were examined microscopically to determine whether defects in the enamel structure were related to the occurrence of systemic ailments. In more than 70% of the cases there was a positive correlation between the time of formation of a band of definitely defective enamel and the existence of some systemic disability. In 23% of the patients there were definite defects in the enamel of patients who had no histories of systemic conditions which might have produced enamel defects. In 6% there were enamel changes in patients who had histories of disabilities which had produced enamel changes in other patients.

Deficiencies of Vitamins A, C, D and also of calcium and phosphorus were the commonest cause of defective enamel formation. Enamel defects were also noted in relation to chicken pox, measles, pneumonia, pertussis, intestinal and gastric disturbances and upper respiratory infection in the children and toxemia of pregnancy in the mother, but in practically all instances these conditions were superimposed upon an inadequate nutritional state.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section[1]
January 29, 1945[2]

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1. Etiological factors in dental degeneration: a study in physical anthropology. *Meyer Klatsky, D.D.S., Medical Department of the Workmen's Circle, New York, N. Y.* This is a study of the physical state of modern, civilized man as compared with ancient and primitive people, with particular interest in the masticatory apparatus. The organs of mastication of modern man are undergoing retrogressive and degenerative changes. The jaw bones are smaller, the muscles involved in chewing less developed, the teeth and their investing tissues are more susceptible to disease than those of the primitive. The tendency to ascribe this to dietary and nutritional deficiencies is not borne out by anthropological evidence. There is ample proof to show that, irrespective of his dental organs, civilized man as a whole is of better skeletal structure, higher stature, more developed musculature, more resistant to disease, and has a longer life span than the savage. Civilized man lives under better environmental conditions; he is better nourished than man of the past. His skeletal muscles are exercised more than those of the primitive because he is doing more productive work and employs his organs more than the primitive. There is a reversed condition as far as the masticatory organs are concerned. The primitive used his jaws and masticatory muscles more efficiently than the civilized. He chewed harder, more resistant and more stimulating food; he was not restricted by civilized habits and manners. Lack of function and exercise of his masticatory organs is mainly responsible for the degenerating state of the masticatory apparatus of modern man.

2. The stimulus threshold of the dental pulp and the periodontal membrane produced by electrical means. *Daniel E. Ziskin, D.D.S. and Edward V. Zagarelli, A.B., M.S., D.D.S., School of Dental and Oral Surgery, Columbia University, New York.* In order to compare the validity of recording responses in electric pulp testing by means of current as against volt stimulation, a special apparatus was constructed. The equipment delivered a 60 cycle alternating current, and included a current meter calibrated to read microamperes and a volt meter. 1605 teeth were tested. Of these, 1289 gave non-vital responses. Of the teeth vital pulps 103 (32.6%) gave responses which, when

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[2]Presiding—Daniel E. Ziskin.

measured by standards usually accepted for voltage testing, were well within the "vital" range. When measured by current stimulation at threshold these responses were seen to emanate from the periodontal membrane and not from the pulp. All tests were verified by other means. It was found that the current-reading device was more reliable in every respect when compared with the voltage method. Tooth resistance became an insignificant factor. It was possible to obtain duplicate readings at threshold thus making quantitative evaluations feasible. Errors in technique and mechanical defects in the apparatus were readily perceptible. The normal threshold of stimulation for anterior vital teeth was found to be between 1 and 20 microamperes and for posterior vital teeth between 1 and 30 microamperes. Most teeth with dead pulps which gave a periodontal membrane response did so at readings between 31 and 100 microamperes whereas the remainder of the non-vital series evidenced no reaction at as high as 100 volts (the full output of the machine) and at current readings of from 70 to 350 microamperes.

3. RADIO-ACTIVE STUDIES ON DENTIN AND ENAMEL.[3] *Gerrit Bevelander, Ph.D. and M. H. Amler, A.B.,*[4] *College of Dentistry, New York University, New York.*

4. TRANSPLANTATION OF DEVELOPING TOOTH GERMS IN THE MANDIBLE OF THE CAT. *Harry H. Shapiro, D.M.D., Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York and Bernice L. Maclean, Ph.D., Department of Biological Sciences, Hunter College, New York.* In 1940, we began a series of experiments to test the capacity of the tooth germ to be substituted for that in another animal of the same species. Developing permanent canine or incisor teeth of kittens were transplanted in place of permanent canine or premolar teeth of older animals and, in a few cases, of young animals approximately the same age as the donors. Intra-oral roentgenograms taken at frequent intervals after operation indicated that the implant, if intact, became established in the alveolar socket of the host and continued its development. Injured implants underwent gradual resorption. A decrease in radiopacity during the first three to five weeks after operation was evident in implanted canine germs, but inconspicuous in implanted incisor germs whose crowns were completely formed at the time of operation. Implants persisting beyond five weeks showed a gradual reappearance of opacity.

5. PYORRHEA EXPERIMENTALLY INDUCED IN RATS UNDER A LOW PANTOTHENIC ACID DIET WITH THE ADDITION OF ZINC CARBONATE. *Daniel E. Ziskin, D.D.S., George Stein, M.D., D.M.D., Paul Gross, M.D., and Edith Runne, A.B., M.A., Columbia University, New York.* The heads of rats fed a synthetic diet complete in every respect, but suboptimal in pantothenic acid and containing 0.8% zinc carbonate were examined. This method of demonstrating existence of a toxic effect of zinc by feeding a suboptimal synthetic diet was first used by one of us (P. G.). The diet was started at about the age of weaning and the rats were sacrificed, or died, at the ages of 11 to 22 weeks. Although the animals devel-

[3] Published in the *J. D. Res.* **24**: 45, 1945.
[4] Guest.

oped the clinical features of pantothenic acid deficiency, the condition was precipitated by zinc poisoning as was demonstrated by the control animals maintained on identical diets without zinc carbonate. There were certain skin lesions which were described at a meeting of the Association of January 27, 1944. Since that time decalcified sections of the molar teeth of these animals have been examined. Changes were observed in the interdental tissue, the soft tissue as well as the septal bone. The degree of the change paralleled the length of time the animals were on the experimental diet. After 50 to 80 days, necrosis of the interdental papillae and in some cases of the alveolar crest and the interradicular septum occurred. A characteristic feature of this necrosis was the almost complete absence of an inflammatory demarcation zone adjacent to the viable tissue. This necrosis resembled that observed in the tongue and cheeks of these animals. After 90 to 120 days the pathologic changes were more marked. Deep pockets developed interdentally, as well as interradicularly, presenting a picture of advanced pyorrhea with almost complete exfoliation of the molar tooth in some animals. None of the control animals showed these changes. These findings in no way resemble the reported pyorrhea in rats occurring as a result of impaction of hair or other extraneous material.